

BIOPOLYMERS MODIFIED WITH SUPEROXIDE DISMUTASE MIMICS

CROSS-REFERENCE TO RELATED APPLICATIONS

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This application is a continuation of U.S. Serial  
No. 09/580,007, filed May 26, 2000, which claims priority  
from provisional application number 60/136,298 filed May  
10 27, 1999, which are hereby incorporated by reference in  
their entirety for all purposes.

BACKGROUND OF THE INVENTION

15 The present invention relates to biomaterials  
modified with non-proteinaceous catalysts for the  
dismutation of superoxide, and processes for making such  
materials. This modification may be by covalent  
conjugation, copolymerization, or admixture of the non-  
20 proteinaceous catalysts with the biomaterial. The  
resulting modified biomaterials exhibit a marked decrease  
in inflammatory response and subsequent degradation when  
placed in contact with vertebrate biological systems.

"Biomaterial" is a term given to a wide variety of  
25 materials which are generally considered appropriate for  
use in biological systems, including metals, polymers,  
biopolymers, and ceramics. Also included in the term are  
composites of such materials, such as the polymer-  
hydroxyapatite composite disclosed in U.S. Pat. No.  
30 5,626,863. Biomaterials are used in a variety of medical  
and scientific applications where a man-made implement  
comes into contact with living tissue. Heart valves,  
stents, replacement joints, screws, pacemaker leads,  
blood vessel grafts, sutures and other implanted devices  
35 constitute one major use of biomaterials. Machines which  
handle bodily fluids for return to the patient, such as  
heart/lung and hemodialysis machines, are another  
significant use for biomaterials.

Common metal alloy biomaterials used for implants include titanium alloys, cobalt-chromium-molybdenum alloys, cobalt-chromium-tungsten-nickel alloys and non-magnetic stainless steels (300 series stainless steel).

5 See U.S. Pat. No. 4,775,426. Titanium alloys are frequently used for implants because they have excellent corrosion resistance. However, they have inferior wear characteristics when compared with either cobalt-chromium-molybdenum alloys or 300 series stainless steel.

10 Cobalt-chromium-molybdenum alloys have about the same tensile strength as the titanium alloys, but are generally less corrosion resistant. They also have the further disadvantage of being difficult to work. In contrast, the 300 series stainless steels were developed

15 to provide high-strength properties while maintaining workability. These steels are, however, even less resistant to corrosion and hence more susceptible to corrosion fatigue. See U.S. Pat. No. 4,718,908. Additional examples of biocompatible metals and alloys

20 include tantalum, gold, platinum, iridium, silver, molybdenum, tungsten, inconel and nitinol. Because certain types of implants (artificial joints, artificial bones or artificial tooth roots) require high strength, metallic biomaterials have conventionally been used.

25 However, as mentioned above, certain alloys corrode within the body and, as a result, dissolved metallic ions can produce adverse effects on the surrounding cells and can result in implant breakage.

In an attempt to solve this problem, ceramic

30 biomaterials such as alumina have been used in high-stress applications such as in artificial knee joints. Ceramic biomaterials have an excellent affinity for bone tissue and generally do not corrode in the body. But when used under the load of walking or the like, they may

35 not remain fixed to the bone. In many cases additional surgery is required to secure the loosened implant. This

shortcoming led to the development of bioactive ceramic materials. Bioactive ceramics such as hydroxyapatite and tricalcium phosphate are composed of calcium and phosphate ions (the main constituents of bone) and are readily resorbed by bone tissue to become chemically united with the bone. U.S. Pat. No. 5,397,362. However, bioactive ceramics such as hydroxyapatite and tricalcium phosphate are relatively brittle and can fail under the loads in the human body. This has led in turn to the development of non-calcium phosphate bioactive ceramics with high strength. See U.S. Pat. No. 5,711,763. Additional examples of biocompatible ceramics include zirconia, silica, calcia, magnesia, and titania series materials, as well as the carbide series materials and the nitride series materials.

Polymeric biomaterials are desirable for implants because of their chemical inertness and low friction properties. However, polymers used in orthopedic devices such as hip and knee joints have a tendency for wear and build-up of fine debris, resulting in a painful inflammatory response. Examples of biocompatible polymeric materials include silicone, polyurethane, polyurethane, polyethylene terephthalate, ultra high molecular weight polyethylene, polypropylene, polyester, polyamide, polycarbonate, polyorthoesters, polyesteramides, polysiloxane, polyolefin, polytetrafluoroethylene, polysulfones, polyanhydrides, polyalkylene oxide, polyvinyl halide, polyvinylidene fluoride, acrylic, methacrylic, polyacrylonitrile, vinyl, polyphosphazene, polyethylene-co-acrylic acid, hydrogels and copolymers. Specific applications include the use of polyethylene in hip and knee joint implants and the use of hydrogels in ocular implants. See U.S. Pat. No. 5,836,313. In addition to relatively inert polymeric materials discussed above, certain medical applications require the use of biodegradable polymers for use as

sutures and pins for fracture fixation. These materials serve as a temporary scaffold which is replaced by host tissue as they are degraded. See U.S. Pat. No. 5,766,618. Examples of such biodegradable polymers  
5 include polylactic acid, polyglycolic acid, and polyparadioxanone.

In addition to wholly synthetic polymers, polymers which are naturally produced by organisms have been used in several medical applications. Such polymers,  
10 including polysaccharides such as chitin, cellulose and hyaluronic acid, and proteins such as fibroin, keratin, and collagen, offer unique physical properties in the biological environment, and are also useful when a biodegradable polymer is required. In order to adapt  
15 these polymers for certain uses, many have been chemically modified, such as chitosan and methyl cellulose. These polymers have found niches in a variety of applications. Chitosan is often used to cast semi-permeable films, such as the dialysis membranes in U.S.  
20 Pat. No. 5,885,609. Fibroin (silk protein) has been used as a support member in tissue adhesive compositions, U.S. Pat. No. 5,817,303. Also, esters of hyaluronic acid have been used to create bioabsorbable scaffolding for the regrowth of nerve tissue, U.S. Pat. No. 5,879,359.

25 As is evident from the preceding paragraphs, individual biomaterials have both desirable and undesirable characteristics. Thus, it is common to create medical devices which are composites of various biocompatible materials in order to overcome these  
30 deficiencies. Examples of such composite materials include: the implant material comprising glass fiber and polymer material disclosed in U.S. Pat. No. 5,013,323; the polymeric-hydroxyapatite bone composite disclosed in U.S. Pat. No. 5,766,618; the implant comprising a ceramic  
35 substrate, a thin layer of glass on the substrate and a layer of calcium phosphate over the glass disclosed in

U.S. Pat. No. 5,397,362; and an implant material comprising carbon fibers in a matrix of fused polymeric microparticles. The diverse uses of biomaterials require a range of mechanical and physical properties for particular applications. As medical science advances, many applications will require new and diverse materials which can be safely and effectively used in biological systems.

Biomaterials, especially polymers, have been chemically modified in several ways in order to give them certain biological characteristics. For instance, thrombogenesis has posed a perennial problem for the use of biomaterials in hemodialysis membranes. In order to decrease thrombogenesis, hemodialysis fluid circuit materials have been modified by ionic complexation and interpenetration of heparin, U.S. Pat. No. 5,885,609, and by graft copolymer techniques in which heparin is linked to the backbone polymer by polyethylene oxide, Park, K.D., "Synthesis and Characterization of SPUU-PEO-Heparin Graft Copolymers", J. Polymer. Sci., Vol. 20, p. 1725-37 (1991). Similarly, polymers containing incorporated drugs for elution into the body have been co-implanted with stents in order to prevent restenosis, U.S. Pat. No. 5,871,535.

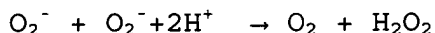
Although most biomaterials in current use are considered non-toxic, implanted biomaterial devices are seen as foreign bodies by the immune system, and so elicit a well characterized inflammatory response. See Gristina, A.G. "Implant Failure and the Immuno-Incompetent Fibro-Inflammatory Zone" In "Clinical Orthopaedics and Related Research" (1994), No. 298, pp. 106-118. This response is evidenced by the increased activity of macrophages, granulocytes, and neutrophils, which attempt to remove the foreign object by the secretion of degradative enzymes and free radicals like superoxide ion ( $O_2^-$ ) to inactivate or decompose the

foreign object. Woven dacron polyester, polyurethane, velcro, polyethylene, and polystyrene were shown to elicit superoxide production from neutrophils by Kaplan, S.S., et al, "Biomaterial-induced alterations of  
5 neutrophil superoxide production" In "Jour.Bio.Mat.Res." (1992), Vol. 26, pp. 1039-1051. To a lesser extent, polysulfone/carbon fiber and polyetherketoneketone/carbon fiber composites were shown to elicit a superoxide response by Moore, R., et al, "A comparison of the  
10 inflammatory potential of particulates derived from two composite materials" In "Jour.Bio.Mat.Res." (1997), Vol. 34, pp. 137-147. Hydroxyapatite, tricalcium phosphate, and aluminum-calcium-phosphorous oxide bioceramics were shown to be degraded by macrophages by Ross, L., et al,  
15 "The Effect of HA, TCP and Alcap Bioceramic Capsules on the Viability of Human Monocyte and Monocyte Derived Macrophages" in "Bio.Sci.Inst." (1996), Vol. 32, pp. 71-79. Similarly, cobalt-chrome alloy beads were degraded by neutrophils in a study by Shanbhag, A., et al,  
20 "Decreased neutrophil respiratory burst on exposure to cobalt-chrome alloy and polystyrene in vitro" In "Jour.Bio.Mat.Res." (1992), Vol. 26, 2, pp. 185-195. Even biomaterials which have been modified to present biologically acceptable molecules, such as heparin, have  
25 been found to elicit an inflammatory response, Borowiec, J.W., et al, "Biomaterial-Dependent Blood Activation During Simulated Extracorporeal Circulation: a Study of Heparin-Coated and Uncoated Circuits", Thorac. Cardiovasc. Surgeon 45 (1997) 295-301. In addition,  
30 chemical modification has posed several difficulties. Because of the unique chemical characteristics of each biomaterial and bioactive molecule, covalent linkage of the desired bioactive molecule to the biomaterial is not always possible. In addition, the activity of many  
35 bioactive molecules, especially proteins, is diminished or extinguished when anchored to a solid substrate.

Finally, the fact that many biologically active substances are heat liable has prevented their use with biomaterials that are molded or worked at high temperatures.

5       The impact of continual attempts by the organism to degrade biomaterial implants can lead to increased morbidity and device failure. In the case of polyurethane pacemaker lead wire coatings, this results in polymer degradation and steady loss of function. In  
10 the use of synthetic vascular grafts, this results in persistent thrombosis, improper healing, and restenosis. As mentioned above, orthopedic devices such as hip and knee joints have a tendency for wear and build-up of fine debris resulting in a painful inflammatory response. In  
15 addition, the surrounding tissue does not properly heal and integrate into the prosthetic device, leading to device loosening and opportunistic bacterial infections. It has been proposed by many researchers that chronic inflammation at the site of implantation leads to the  
20 exhaustion of the macrophages and neutrophils, and an inability to fight off infection.

Superoxide anions are normally removed in biological systems by the formation of hydrogen peroxide and oxygen in the following reaction (hereinafter referred to as  
25 dismutation):



This reaction is catalyzed *in vivo* by the ubiquitous superoxide dismutase enzyme. Several non-proteinaceous catalysts which mimic this superoxide dismutating  
30 activity have been discovered. A particularly effective family of non-proteinaceous catalysts for the dismutation of superoxide consists of the manganese(II), manganese(III), iron(II) or iron(III) complexes of nitrogen-containing fifteen-membered macrocyclic ligands  
35 which catalyze the conversion of superoxide into oxygen and hydrogen peroxide, described in U.S. Patents Nos.

5,874,421 and 5,637,578, all of which are incorporated herein by reference. See also Weiss, R.H., et al, "Manganese(II)-Based Superoxide Dismutase Mimetics: Rational Drug Design of Artificial Enzymes", (1996) Drugs of the Future 21, 383-389; and Riley, D.P., et al, "Rational Design of Synthetic Enzymes and Their Potential Utility as Human Pharmaceuticals" (1997) in CatTech, I, 41. These mimics of superoxide dismutase have been shown to have a variety of therapeutic effects, including anti-inflammatory activity. See Weiss, R.H., et al, "Therapeutic Aspects of Manganese (II)-Based Superoxide Dismutase Mimics" In "Inorganic Chemistry in Medicine", (Farrell, N., Ed.), Royal Society of Chemistry, in Press; Weiss, R.H., et al, "Manganese-Based Superoxide Dismutase Mimics: Design, Discovery and Pharmacologic Efficacies" (1995) In "The Oxygen Paradox (Davies, K.J.A., and Ursini, F., Eds.) pp. 641-651, CLEUP University Press, Padova, Italy; Weiss, R.H., et al, "Manganese-Based Superoxide Dismutase Mimetic Inhibit Neutrophil Infiltration In Vitro", J.Biol.Chem., 271, 26149 (1996); and Hardy, M.M., et al, "Superoxide Dismutase Mimetics Inhibit Neutrophil-Mediated Human Aortic Endothelial Cell Injury In Vitro", (1994) J.Biol.Chem. 269, 18535-18540. Other non-proteinaceous catalysts which have been shown to have superoxide dismutating activity are the salen-transition metal cation complexes, described in U.S. Patent No. 5,696,109, and complexes of porphyrins with iron and manganese cations.

### SUMMARY OF THE INVENTION

Applicants have discovered that the modification of biomaterials with non-proteinaceous catalysts for the dismutation of superoxide greatly improves the biomaterial's resistance to degradation and reduces the inflammatory response. Thus, the present invention is directed to biomaterials which have been modified with



non-proteinaceous catalysts for the dismutation of superoxide, or precursor ligands of non-proteinaceous catalysts for the dismutation of superoxide.

5 The present invention is directed to biomaterials which have been modified with non-proteinaceous catalysts for the dismutation of superoxide, or precursor ligands of a non-proteinaceous catalyst for the dismutation of superoxide, by utilizing methods of physical association, such as surface covalent conjugation, copolymerization, and physical admixing. The present invention is also  
10 directed to biomaterials modified with non-proteinaceous catalysts for the dismutation of superoxide wherein one or more of these methods has been used to modify the biomaterial.

15 A variety of biomaterials are appropriate for modification in the present invention. Because the non-proteinaceous catalysts for the dismutation of superoxide are suitable for use in a range of methods for physically associating the catalyst with the biomaterial, almost any  
20 biomaterial may be modified according to the present invention. The biomaterial to be modified may be any biologically compatible metal, ceramic, polymer, biopolymer, biologically derived material, or a composite thereof. Thus, the present invention is further directed  
25 towards any of the above biomaterials modified with non-proteinaceous catalysts for the dismutation of superoxide.

As previously mentioned, the non-proteinaceous catalysts for the dismutation of superoxide for use in  
30 the present invention comprise an organic ligand and a transition metal cation. Particularly preferred catalysts are manganese and iron chelates of pentaazacyclopentadecane compounds (hereinafter referred to as "PACPeD catalysts"). Also suitable for use in the  
35 present invention are the salen complexes of manganese and iron disclosed in U.S. Patent No. 5,696,109, and

iron or manganese porphyrins, such as  $\text{Mn}^{\text{III}}$  tetrakis(4-N-methylpyridyl)porphyrin,  $\text{Mn}^{\text{III}}$  tetrakis-o-(4-N-methylisonicotinamidophenyl)porphyrin,  $\text{Mn}^{\text{III}}$  tetrakis(4-N-N-N-trimethylanilinium)porphyrin,  $\text{Mn}^{\text{III}}$  tetrakis(1-methyl-  
5 4-pyridyl)porphyrin,  $\text{Mn}^{\text{III}}$  tetrakis(4-benzoic acid)porphyrin,  $\text{Mn}^{\text{II}}$  octabromo-meso-tetrakis(N-methylpyridinium-4-yl)porphyrin,  $\text{Fe}^{\text{III}}$  tetrakis(4-N-methylpyridyl)porphyrin, and  $\text{Fe}^{\text{III}}$  tetrakis-o-(4-N-methylisonicotinamidophenyl)porphyrin. These non-  
10 proteinaceous catalysts for the dismutation of superoxide also preferably contain a reactive moiety when the methods of surface covalent conjugation or copolymerization are used to modify the biomaterial. Thus, the present invention is directed to biomaterials  
15 which have been modified with any of the above non-proteinaceous catalysts for the dismutation of superoxide. In addition, as sometimes it is advantageous to add the chelated transition metal ion after the biomaterial has been modified, the present invention is  
20 also directed to biomaterials which have been modified with the precursor ligand of any of the above non-proteinaceous catalysts.

The present invention is also directed to processes for producing biomaterials modified by surface covalent  
25 conjugation with at least one non-proteinaceous catalyst for the dismutation of superoxide or at least one precursor ligand of a non-proteinaceous catalyst for the dismutation of superoxide, the process comprising:

- a. providing at least one reactive functional group on a surface of the biomaterial to be modified;
- 5 b. providing at least one complementary reactive functional group on the non-proteinaceous catalyst for the dismutation of superoxide or on the precursor ligand; and
- c. conjugating the non-proteinaceous catalyst for the dismutation of superoxide or the precursor  
10 ligand with the surface of the biomaterial through at least one covalent bond.

The non-proteinaceous catalyst for the dismutation of superoxide or the precursor ligand can be covalently bound directly to the surface of the biomaterial, or  
15 bound to the surface through a linker molecule. Thus, the present invention is also directed to the above process further comprising providing a bi-functional linker molecule.

The present invention is also directed to a process  
20 for producing a biomaterial modified by co-polymerization with at least one non-proteinaceous catalyst for the dismutation of superoxide or at least one ligand precursor of a non-proteinaceous catalyst for the dismutation of superoxide, the process comprising:

- 25 a. providing at least one monomer;
- b. providing at least one non-proteinaceous catalyst for the dismutation of superoxide or at least one ligand precursor of a non-proteinaceous catalyst for the  
30 dismutation of superoxide containing at least one functional group capable of reaction with the monomer and also containing at least one functional group capable of propagation of the polymerization reaction,
- 35 c. copolymerizing the monomers and the non-proteinaceous catalyst for the

dismutation of superoxide or the ligand precursor in a polymerization reaction.

The present invention is also directed to a process for producing a biomaterial modified by admixture with at least one non-proteinaceous catalyst for the dismutation of superoxide or a precursor ligand of a non-proteinaceous catalyst for the dismutation of superoxide, the process comprising:

- a. providing at least one unmodified biomaterial;
- b. providing at least one non-proteinaceous catalyst for the dismutation of superoxide or at least one ligand precursor of a non-proteinaceous catalyst for the dismutation of superoxide; and
- c. admixing the unmodified biomaterial and the non-proteinaceous catalyst for the dismutation of superoxide or the ligand precursor.

In addition, the present invention is also directed to a novel method of synthesizing PACPeD catalysts by using manganese or other transition metal ions as a template for cyclization the ligand.

The present invention is also directed to a biocompatible article comprising a biomaterial modified with at least one non-proteinaceous catalyst for the dismutation of superoxide or a ligand precursor of a non-proteinaceous catalyst for the dismutation of superoxide, wherein the catalyst or ligand precursor is presented on a surface of the article. The invention is also directed to the use of the biomaterials of the present invention in a stent, a vascular graft fabric, a nerve growth channel, a cardiac lead wire, or other medical devices for implantation in or contact with the body or bodily fluids.

#### BRIEF DESCRIPTION OF DRAWINGS AND DEFINITIONS

### Drawings

FIGURE 1: An electron micrograph of the surface of a control disk of poly(etherurethane urea) which has not been implanted.

5        FIGURE 2: An electron micrograph of the surface of a control disk of poly(etherurethane urea) (not conjugated with a non-proteinaceous catalyst for the dismutation of superoxide) which has been implanted in a rat for 28 days.

10       FIGURE 3: An electron micrograph of the surface of a poly(etherurethane urea) disc which has been conjugated with Compound 43 and which has been implanted in a rat for 28 days.

15       FIGURE 4: A comparison of capsules formed around polypropylene fibers which have been implanted into a rat. A) a control fiber, made of polypropylene which has not been admixed with a non-proteinaceous catalyst for the dismutation of superoxide; B) a fiber made of polypropylene which has been admixed with Compound 54, 2%  
20 by weight.

FIGURE 5: A comparison of capsules formed around disks of polyethylene which have been implanted in a rat for 3 days. A) control disk, not conjugated with a non-proteinaceous catalyst; B) a disk conjugated with  
25 Compound 43, 0.06% by weight; C) a disk conjugated with Compound 43, 1.1% by weight.

FIGURE 6: A comparison of capsules formed around disks of polyethylene which have been implanted in a rat for 28 days. A) control disk, not conjugated with a non-proteinaceous catalyst; B) a disk conjugated with  
30 Compound 43, 0.06% by weight; C) a disk conjugated with Compound 43, 1.1% by weight.

FIGURE 7: A graphical comparison of the capsule thickness and number of giant cells in the capsule for  
35 polyethylene disks conjugated with Compound 43, 0.06% by weight, and polyethylene disks conjugated with Compound 43, 1.1% by weight, after implantation for 28 days.

FIGURE 8: A comparison of capsules formed around disks of poly(etherurethane urea) which have been implanted in a rat for 28 days. A) control disk, not conjugated with a non-proteinaceous catalyst; B) a disk  
 5 conjugated with Compound 43, 0.6% by weight; C) a disc conjugated with Compound 43, 3.0% by weight.

FIGURE 9: A comparison of capsules formed around disks of tantalum which have been implanted in a rat for 3 days. A) control disk, conjugated only with the silyl  
 10 linker; B) a disk conjugated with Compound 43 via the silyl linker.

FIGURE 10: A comparison of capsules formed around disks of tantalum which have been implanted in a rat for 28 days. A) control disk, conjugated only with the silyl  
 15 linker; B) a disk conjugated with Compound 43 via the silyl linker.

\_\_\_\_ FIGURE 11: A drawing of the unwound wire used to make the stent of Example 26.

FIGURE 12: A close up of the bends and "eyes" in the  
 20 wire of Figure 11

FIGURE 13: A side view drawing of the helically wound stent, fully expanded.

FIGURE 14: A cross-section of the helically wound stent.

25 FIGURE 15: A side view drawing of the helically wound stent, compressed.

FIGURE 16: A detailed view of the helically wound stent, showing the angle of the helix (  $\exists$  ) and the angle between the zig-zags of the stent wire (  $\nabla$  ).

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### Definitions

As utilized herein, the term "biomaterial" includes any generally non-toxic material commonly used in applications where contact with biological systems is  
 35 expected. Examples of biomaterials include: metals such as stainless steel, tantalum, titanium, nitinol, gold, platinum, inconel, iridium, silver, molybdenum, tungsten,

nickel, chromium, vanadium, and alloys comprising any of the foregoing metals and alloys; ceramics such as hydroxyapatite, tricalcium phosphate, and aluminum-calcium-phosphorus oxide; polymers such as polyurethane, polyurethane, polyalkylene glycols, polyethylene terephthalate, ultra high molecular weight polyethylenes, polypropylene, polyesters, polyamides, polycarbonates, polyorthoesters, polyesteramides, polysiloxanes, polyolefins, polytetrafluoroethylenes, polysulfones, polyanhydrides, polyalkylene oxides, polyvinyl halides, polyvinylidene halides, acrylics, methacrylics, polyacrylonitriles, polyvinyls, polyphosphazenes, polyethylene-co-acrylic acid, silicones, block copolymers of any of the foregoing polymers, random copolymers of any of the foregoing polymers, graft copolymers of any of the foregoing polymers, crosslinked polymers of any of the foregoing polymers, hydrogels, and mixtures of any of the foregoing polymers; biopolymers such as chitin, chitosan, cellulose, methyl cellulose, hyaluronic acid, keratin, fibroin, collagen, elastin, and saccharide polymers; biologically derived materials such as fixed tissues, and composites of such materials.

"Biocompatible" articles are fabricated out of biomaterials. As used herein, the term "biomaterial" is not meant to encompass drugs and biologically active molecules such as steroids, di-saccharides and short chain polysaccharides, fatty acids, amino acids, antibodies, vitamins, lipids, phospholipids, phosphates, phosphonates, nucleic acids, enzymes, enzyme substrates, enzyme inhibitors, or enzyme receptor substrates.

The term "non-proteinaceous catalysts for the dismutation of superoxide" means a low-molecular-weight catalyst for the conversion of superoxide anions into hydrogen peroxide and molecular oxygen. These catalysts commonly consist of an organic ligand and a chelated transition metal ion, preferably manganese or iron. The term may include catalysts containing short-chain

polypeptides (under 15 amino acids), or macrocyclic structures derived from amino acids, as the organic ligand. The term explicitly excludes a superoxide dismutase enzyme obtained from any species.

5       The term "precursor ligand" means the organic ligand of a non-proteinaceous catalyst for the dismutation of superoxide without the chelated transition metal cation.

          The term "biopolymer" means a polymer which can be produced in a living system or synthetically out of amino  
10   acids, saccharides, or other typical biological monomers. The term also encompasses derivatives of these biological polymers. Examples of biopolymers include chitin, chitosan, cellulose, methyl cellulose, hyaluronic acid, keratin, fibroin, collagen, and elastin.

15       The term "biologically derived material" means biological tissue which has been chemically modified for implantation into a new host, such as fixed heart valves and blood vessels.

          The term "modification" means any method by which a  
20   physical association may be effected between a biomaterial and a non-proteinaceous catalyst for the dismutation of superoxide, whereby the non-proteinaceous catalyst becomes integrated into or onto the biomaterial. Modification may be effected by surface covalent  
25   conjugation, copolymerization, admixture, or by other methods. When modification is achieved by admixture, it is understood that the non-proteinaceous catalyst is in the same phase as at least a part of the biomaterial that is modified.

30       The term "surface covalent conjugation" means that the non-proteinaceous catalyst is bound through at least one covalent bond to the surface of a biomaterial. The term encompasses conjugation via a direct covalent bond between the non-proteinaceous catalyst and the surface,  
35   as well as an indirect bond which includes a linker molecule between the non-proteinaceous catalyst and the surface of the biomaterial.

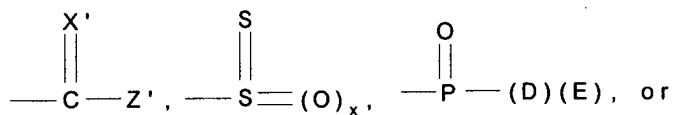


The term "linker" means any molecule with at least two functional groups which can be used to "link" one molecule to another. Examples of linkers include low molecular weight polyethylene glycol, hexamethyl  
5 di(imidi)-isocyanate, silyl chloride, and polyglycine.

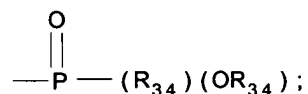
The term "copolymerization" means that the non-proteinaceous catalyst is copolymerized with the monomer which forms the biomaterial, and thus integrated into the polymer chain of the modified biomaterial.

10 The term "inflammatory response" means that the material elicits the inflammation of the surrounding tissues and the production of degradative enzymes and reactive molecular species when exposed to biological systems.

15 The term "substituted" means that the described moiety has one or more of the following substituents:  
(1)  $-NR_{30}R_{31}$  wherein  $R_{30}$  and  $R_{31}$  are independently selected from hydrogen, alkyl, aryl or aralkyl; or  $R_{30}$  is hydrogen, alkyl, aryl or aralkyl and  $R_{31}$  is selected from the group  
20 consisting of  $-NR_{32}R_{33}$ ,  $-OH$ ,  $-OR_{34}$ ,

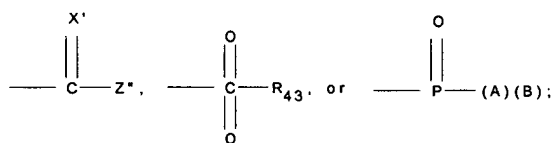


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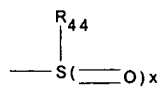
- 10 wherein  $R_{32}$  and  $R_{33}$  are independently hydrogen, alkyl, aryl or acyl,  $R_{34}$  is alkyl, aryl or alkaryl,  $Z'$  is hydrogen, alkyl, aryl, alkaryl,  $\text{---OR}_{34}$ ,  $\text{---SR}_{34}$  or  $\text{---NR}_{40}R_{41}$ .  $R_{37}$  is alkyl, aryl or alkaryl,  $X'$  is oxygen or sulfur, and  $R_{38}$  and  $R_{39}$  are independently selected from hydrogen, alkyl, or aryl;
- 15 (2)  $\text{---SR}_{42}$  wherein  $R_{42}$  is hydrogen, alkyl, aryl, alkaryl,  $\text{---SR}_{34}$ ,  $\text{---NR}_{32}R_{33}$ ,

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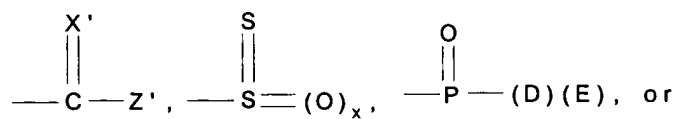


- wherein  $R_{43}$  is  $\text{---OH}$ ,  $\text{---OR}_{34}$  or  $\text{---NR}_{32}R_{33}$ , and A and B are
- 25 independently  $\text{---OR}_{34}$ ,  $\text{---SR}_{34}$  or  $\text{---NR}_{32}R_{33}$

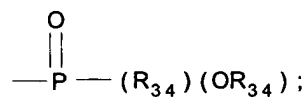
- 30 (3) wherein  $x$  is 1 or 2, and  $R_{44}$  is halide, alkyl, aryl, alkaryl,  $\text{---OH}$ ,  $\text{---OR}_{34}$  or  $\text{---NR}_{32}R_{33}$ ;
- (4)  $\text{---OR}_{45}$  wherein  $R_{45}$  is hydrogen, alkyl, aryl, alkaryl,  $\text{---NR}_{32}R_{33}$ ,



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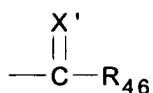
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wherein D and E are independently  $-OR_{34}$  or  $-NR_{32}R_{33}$ ;

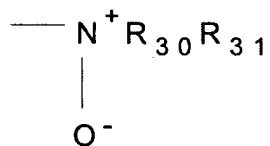
15

(5)



20 wherein  $R_{46}$  is halide,  $-OH$ ,  $-SH$ ,  $-OR_{34}$ ,  $-SR_{34}$  or  $-NR_{32}R_{33}$ ;

(6) amine oxides of the formula

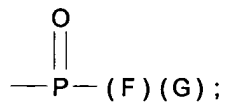


25

provided  $R_{30}$  and  $R_{31}$  are not hydrogen;

(7)

30



35

wherein F and G are independently  $-OH$ ,  $-SH$ ,  $-OR_{34}$ ,  $-SR_{34}$  or  $-NR_{32}R_{33}$ ;

(8)  $-O-(-(CH_2)_a-O)_b-R_{10}$  wherein  $R_{10}$  is hydrogen or alkyl, and a and b are integers independently selected from 1 + 6;

(9) halogen, cyano, nitro or azido; or

5 (10) aryl, heteroaryl, alkynyl, or alkenyl.

Alkyl, aryl and alkaryl groups on the substituents of the above-defined alkyl groups may contain one or more additional substituents, but are preferably unsubstituted.

10 The term "functional group" means a group capable of reacting with another functional group to form a covalent bond. Functional groups preferably used in the present invention include acid halide ( $XCO-$  wherein  $X = Cl, F, Br, I$ ), amino ( $H_2N-$ ), isocyanate ( $OCN-$ ), mercapto ( $HS-$ ),  
 15 glycidyl ( $H_2COCH-$ ), carboxyl ( $HOCO-$ ), hydroxy ( $HO-$ ), and chloromethyl ( $ClH_2C-$ ), silyl or silyl chloride, and substituted or unsubstituted alkenyl, alkynyl, aryl, and heteroaryl.

The term "alkyl", alone or in combination, means a  
 20 straight-chain or branched-chain alkyl radical containing from 1 to about 22 carbon atoms, preferably from about 1 to about 18 carbon atoms, and most preferably from about 1 to about 12 carbon atoms. Examples of such radicals include, but are not limited to, methyl, ethyl, n-propyl,  
 25 isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl, hexyl, octyl, nonyl, decyl, dodecyl, tetradecyl, hexadecyl, octadecyl and eicosyl.

The term "alkenyl", alone or in combination, means an alkyl radical having one or more double bonds.  
 30 Examples of such alkenyl radicals include, but are not limited to, ethenyl, propenyl, 1-butenyl, cis-2-butenyl, trans-2-butenyl, iso-butylenyl, cis-2-pentenyl, trans-2-pentenyl, 3-methyl-1-butenyl, 2,3-dimethyl-2-butenyl, 1-pentenyl, 1-hexenyl, 1-octenyl, decenyl, dodecenyl,  
 35 tetradecenyl, hexadecenyl, cis- and trans-9-octadecenyl, 1,3-pentadienyl, 2,4-pentadienyl, 2,3-pentadienyl, 1,3-

hexadienyl, 2,4-hexadienyl, 5,8,11,14-eicosatetraenyl, and 9,12,15-octadecatrienyl.

The term "alkynyl", alone or in combination, means an alkyl radical having one or more triple bonds.

- 5 Examples of such alkynyl groups include, but are not limited to, ethynyl, propynyl (propargyl), 1-butylnyl, 1-octynyl, 9-octadecynyl, 1,3-pentadiynyl, 2,4-pentadiynyl, 1,3-hexadiynyl, and 2,4-hexadiynyl.

- The term "cycloalkyl", alone or in combination means  
10 a cycloalkyl radical containing from 3 to about 10, preferably from 3 to about 8, and most preferably from 3 to about 6, carbon atoms. Examples of such cycloalkyl radicals include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl,  
15 cyclooctyl, and perhydronaphthyl.

- The term "cycloalkylalkyl" means an alkyl radical as defined above which is substituted by a cycloalkyl radical as defined above. Examples of cycloalkylalkyl radicals include, but are not limited to,  
20 cyclohexylmethyl, cyclopentylmethyl, (4-isopropylcyclohexyl)methyl, (4-*t*-butyl-cyclohexyl)methyl, 3-cyclohexylpropyl, 2-cyclohexylmethylpentyl, 3-cyclopentylmethylhexyl, 1-(4-neopentylcyclohexyl)methylhexyl, and 1-(4-isopropylcyclohexyl)methylheptyl.  
25

- The term "cycloalkylcycloalkyl" means a cycloalkyl radical as defined above which is substituted by another cycloalkyl radical as defined above. Examples of cycloalkylcycloalkyl radicals include, but are not  
30 limited to, cyclohexylcyclopentyl and cyclohexylcyclohexyl.

- The term "cycloalkenyl", alone or in combination, means a cycloalkyl radical having one or more double bonds. Examples of cycloalkenyl radicals include, but are  
35 not limited to, cyclopentenyl, cyclohexenyl, cyclooctenyl, cyclopentadienyl, cyclohexadienyl and cyclooctadienyl.

The term "cycloalkenylalkyl" means an alkyl radical as defined above which is substituted by a cycloalkenyl radical as defined above. Examples of cycloalkenylalkyl radicals include, but are not limited to, 2-cyclohexen-1-ylmethyl, 1-cyclopenten-1-ylmethyl, 2-(1-cyclohexen-1-yl)ethyl, 3-(1-cyclopenten-1-yl)propyl, 1-(1-cyclohexen-1-ylmethyl)pentyl, 1-(1-cyclopenten-1-yl)hexyl, 6-(1-cyclohexen-1-yl)hexyl, 1-(1-cyclopenten-1-yl)nonyl and 1-(1-cyclohexen-1-yl)nonyl.

10       The terms "alkylcycloalkyl" and "alkenylcycloalkyl" mean a cycloalkyl radical as defined above which is substituted by an alkyl or alkenyl radical as defined above. Examples of alkylcycloalkyl and alkenylcycloalkyl radicals include, but are not limited to, 2-ethylcyclobutyl, 1-methylcyclopentyl, 1-hexylcyclopentyl, 1-methylcyclohexyl, 1-(9-octadecenyl)cyclopentyl and 1-(9-octadecenyl)cyclohexyl.

      The terms "alkylcycloalkenyl" and "alkenylcycloalkenyl" means a cycloalkenyl radical as defined above which is substituted by an alkyl or alkenyl radical as defined above. Examples of alkylcycloalkenyl and alkenylcycloalkenyl radicals include, but are not limited to, 1-methyl-2-cyclopentenyl, 1-hexyl-2-cyclopentenyl, 1-ethyl-2-cyclohexenyl, 1-butyl-2-cyclohexenyl, 1-(9-octadecenyl)-2-cyclohexenyl and 1-(2-pentenyl)-2-cyclohexenyl.

      The term "aryl", alone or in combination, means a phenyl or naphthyl radical which optionally carries one or more substituents selected from alkyl, cycloalkyl, cycloalkenyl, aryl, heterocycle, alkoxyaryl, alkaryl, alkoxy, halogen, hydroxy, amine, cyano, nitro, alkylthio, phenoxy, ether, trifluoromethyl and the like, such as phenyl, p-tolyl, 4-methoxyphenyl, 4-(tert-butoxy)phenyl, 4-fluorophenyl, 4-chlorophenyl, 4-hydroxyphenyl, 1-naphthyl, 2-naphthyl, and the like.

      The term "aralkyl", alone or in combination, means an alkyl or cycloalkyl radical as defined above in which

one hydrogen atom is replaced by an aryl radical as defined above, such as benzyl, 2-phenylethyl, and the like.

The term "heterocyclic" means ring structures  
 5 containing at least one other kind of atom, in addition to carbon, in the ring. The most common of the other kinds of atoms include nitrogen, oxygen and sulfur. Examples of heterocyclics include, but are not limited to, pyrrolidinyl, piperidyl, imidazolidinyl,  
 10 tetrahydrofuryl, tetrahydrothienyl, furyl, thienyl, pyridyl, quinolyl, isoquinolyl, pyridazinyl, pyrazinyl, indolyl, imidazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, benzoxadiazolyl, benzothiadiazolyl, triazolyl and tetrazolyl groups.

15 The term "saturated, partially saturated or unsaturated cyclic" means fused ring structures in which 2 carbons of the ring are also part of the fifteen-membered macrocyclic ligand. The ring structure can contain 3 to 20 carbon atoms, preferably 5 to 10 carbon  
 20 atoms, and can also contain one or more other kinds of atoms in addition to carbon. The most common of the other kinds of atoms include nitrogen, oxygen and sulfur. The ring structure can also contain more than one ring.

The term "saturated, partially saturated or  
 25 unsaturated ring structure" means a ring structure in which one carbon of the ring is also part of the fifteen-membered macrocyclic ligand. The ring structure can contain 3 to 20, preferably 5 to 10, carbon atoms and can also contain nitrogen, oxygen and/or sulfur atoms.

30 The term "nitrogen containing heterocycle" means ring structures in which 2 carbons and a nitrogen of the ring are also part of the fifteen-membered macrocyclic ligand. The ring structure can contain 2 to 20, preferably 4 to 10, carbon atoms, can be substituted or  
 35 unsubstituted, partially or fully unsaturated or saturated, and can also contain nitrogen, oxygen and/or

sulfur atoms in the portion of the ring which is not also part of the fifteen-membered macrocyclic ligand.

The term "organic acid anion" refers to carboxylic acid anions having from about 1 to about 18 carbon atoms.

5       The term "halide" means chloride, fluoride, iodide, or bromide.

As used herein, "R" groups means all of the R groups attached to the carbon atoms of the macrocycle, i.e., R, R', R<sub>1</sub>, R'<sub>1</sub>, R<sub>2</sub>, R'<sub>2</sub>, R<sub>3</sub>, R'<sub>3</sub>, R<sub>4</sub>, R'<sub>4</sub>, R<sub>5</sub>, R'<sub>5</sub>, R<sub>6</sub>, R'<sub>6</sub>, R<sub>7</sub>,  
10 R'<sub>7</sub>, R<sub>8</sub>, R'<sub>8</sub>, R<sub>9</sub>.

All references cited herein are explicitly incorporated by reference.

#### DETAILED DESCRIPTION OF THE INVENTION

15

The present invention concerns novel modified biomaterials and methods for the production of such materials. Prior to applicants' invention, it was not known that non-proteinaceous catalysts for the  
20 dismutation of superoxide could be immobilized on the surface of a biomaterial and still retain their catalytic function and exhibit an anti-inflammatory effect. However, applicants have found that these catalysts can be efficaciously immobilized on biomaterial surfaces and  
25 still retain superoxide dismutating ability, as shown by Example 23. Applicants have also found that these modified biomaterials have greatly improved durability and decreased inflammatory response when exposed to biological systems, such as the rat model in Examples 21  
30 and 22.



Biomaterials and Non-Proteinaceous Catalysts for the  
Dismutation of Superoxide for Use in the Present  
Invention

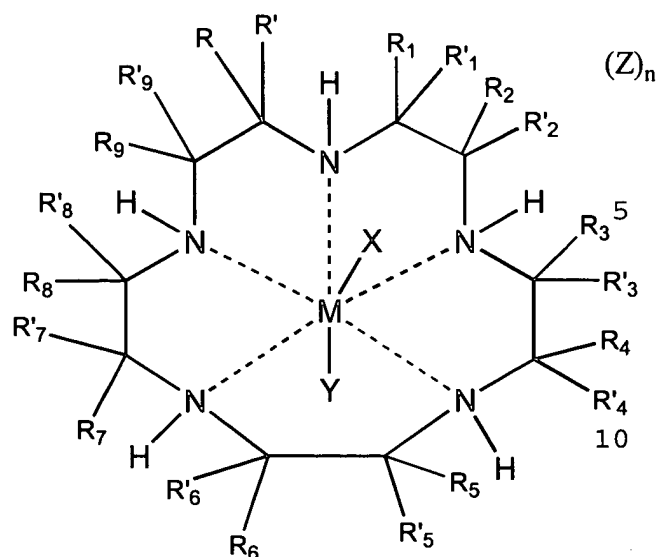
A variety of biomaterials are appropriate for  
5 modification in the present invention. The biomaterial to  
be modified can be any biologically compatible metal,  
ceramic, polymer, biopolymer, or a composite thereof.  
Metals suitable for use in the present invention include  
stainless steel, tantalum, titanium, nitinol, gold,  
10 platinum, inconel, iridium, silver, molybdenum, tungsten,  
nickel, chromium, vanadium, and alloys comprising any of  
the foregoing metals and alloys. Ceramics suitable for  
use in the present invention include hydroxyapatite,  
tricalcium phosphate, and aluminum-calcium-phosphorus  
15 oxide. Polymers suitable for use in the present  
invention include polyurethane, polyureaurethane,  
polyalkylene glycols, polyethylene terephthalate, ultra  
high molecular weight polyethylenes, polypropylene,  
polyesters, polyamides, polycarbonates, polyorthoesters,  
20 polyesteramides, polysiloxanes, polyolefins,  
polytetrafluoroethylenes, polysulfones, polyanhydrides,  
polyalkylene oxides, polyvinyl halides, polyvinylidene  
halides, acrylics, methacrylics, polyacrylonitriles,  
polyvinyls, polyphosphazenes, polyethylene-co-acrylic  
25 acid, silicones, block copolymers of any of the foregoing  
polymers, random copolymers of any of the foregoing  
polymers, graft copolymers of any of the foregoing  
polymers, crosslinked polymers of any of the foregoing  
polymers, hydrogels, and mixtures of any of the foregoing  
30 polymers. Biopolymers suitable for use in the present  
invention are chitin, chitosan, cellulose, methyl  
cellulose, hyaluronic acid, keratin, fibroin, collagen,  
elastin, and saccharide polymers. Composite materials  
which may be used in the present invention comprise a  
35 relatively inelastic phase such as carbon, hydroxy  
apatite, tricalcium phosphate, silicates, ceramics, or

metals, and a relatively elastic phase such as a polymer or biopolymer.

Where the method used to modify the biomaterial is surface covalent conjugation, the unmodified biomaterial should contain, or be chemically derivatized to contain, a reactive moiety. Preferred reactive moieties include acid halide (XCO- wherein X= Cl, F, Br, I), amino (H<sub>2</sub>N-), isocyanate (OCN-), mercapto (HS-), glycidyl (H<sub>2</sub>COCH-), carboxyl (HOCO-), hydroxy (HO-), and chloromethyl (ClH<sub>2</sub>C-), silyl or silyl chloride, and substituted or unsubstituted alkenyl, alkynyl, aryl, and heteroaryl moieties.

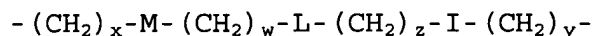
Applicants have discovered that these compounds, especially the preferred pentaaza non-proteinaceous catalysts, will survive a wide range of chemical reactions and processing conditions including extreme chemical and thermal conditions. Particularly, the PACPeD catalysts have been demonstrated by the applicants to be stable at temperatures up to about 350EC, and at pH of about 4. Additionally, the PACPeD's are soluble in a wide range of solvents, including water, methanol, ethanol, methylene chloride, DMSO, DMF, and DMAC, and are partially soluble in toluene and acetonitrile. By adding polar or non-polar substituents at the R group positions on the PACPeD or other non-proteinaceous catalysts, applicants have improved their solubility in specific solvents for particular reactions, and for use with particular biomaterials. As illustrated by Table 1 below, several reactive functional groups may be added as pendant moieties without detrimentally affecting the catalyst's superoxide dismutating ability.

The non-proteinaceous catalysts for the dismutation of superoxide for use in the present invention preferably comprise an organic ligand and a transition metal cation. Particularly preferred catalysts are manganese and iron chelates of pentaazacyclopentadecane compounds, which can be represented by the following formula:



- 15 wherein  $M$  is a cation of a transition metal, preferably manganese or iron; wherein  $R, R', R_1, R'_1, R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8, R'_8, R_9, R'_9$  independently represent hydrogen, or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cycloalkylcycloalkyl, cycloalkenylalkyl, alkylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkyl, alkenylcycloalkenyl, heterocyclic, aryl and aralkyl radicals;  $R_1$  or  $R'_1$  and  $R_2$  or  $R'_2, R_3$  or  $R'_3$  and  $R_4$  or  $R'_4, R_5$  or  $R'_5$  and  $R_6$  or  $R'_6, R_7$  or  $R'_7$  and  $R_8$  or  $R'_8$ , and  $R_9$  or  $R'_9$  and  $R$  or  $R'$  together with the carbon atoms to which they are attached independently form a substituted or unsubstituted, saturated, partially saturated or unsaturated cyclic or heterocyclic having 3 to 20 carbon atoms;  $R$  or  $R'$  and  $R_1$  or  $R'_1, R_2$  or  $R'_2$  and  $R_3$  or  $R'_3, R_4$  or  $R'_4$  and  $R_5$  or  $R'_5, R_6$  or  $R'_6$  and  $R_7$  or  $R'_7$ , and  $R_8$  or  $R'_8$  and  $R_9$  or  $R'_9$  together with the carbon atoms to which they are attached independently form a substituted or unsubstituted nitrogen containing heterocycle having 2 to 20 carbon atoms, provided that when the nitrogen containing heterocycle is an aromatic heterocycle which does not contain a hydrogen attached to the nitrogen, the hydrogen attached to the nitrogen as

shown in the above formula, which nitrogen is also in the macrocyclic ligand or complex, and the R groups attached to the included carbon atoms of the macrocycle are absent; R and R', R<sub>1</sub> and R'<sub>1</sub>, R<sub>2</sub> and R'<sub>2</sub>, R<sub>3</sub> and R'<sub>3</sub>, R<sub>4</sub> and R'<sub>4</sub>, R<sub>5</sub> and R'<sub>5</sub>, R<sub>6</sub> and R'<sub>6</sub>, R<sub>7</sub> and R'<sub>7</sub>, R<sub>8</sub> and R'<sub>8</sub>, and R<sub>9</sub> and R'<sub>9</sub>, together with the carbon atom to which they are attached independently form a saturated, partially saturated, or unsaturated cyclic or heterocyclic having 3 to 20 carbon atoms; and one of R, R', R<sub>1</sub>, R'<sub>1</sub>, R<sub>2</sub>, R'<sub>2</sub>, R<sub>3</sub>, R'<sub>3</sub>, R<sub>4</sub>, R'<sub>4</sub>, R<sub>5</sub>, R'<sub>5</sub>, R<sub>6</sub>, R'<sub>6</sub>, R<sub>7</sub>, R'<sub>7</sub>, R<sub>8</sub>, R'<sub>8</sub>, R<sub>9</sub>, and R'<sub>9</sub> together with a different one of R, R', R<sub>1</sub>, R'<sub>1</sub>, R<sub>2</sub>, R'<sub>2</sub>, R<sub>3</sub>, R'<sub>3</sub>, R<sub>4</sub>, R'<sub>4</sub>, R<sub>5</sub>, R'<sub>5</sub>, R<sub>6</sub>, R'<sub>6</sub>, R<sub>7</sub>, R'<sub>7</sub>, R<sub>8</sub>, R'<sub>8</sub>, R<sub>9</sub>, and R'<sub>9</sub> which is attached to a different carbon atom in the macrocyclic ligand may be bound to form a strap represented by the formula



wherein w, x, y and z independently are integers from 0 to 10 and M, L and J are independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, cycloalkyl, heteroaryl, alkaryl, alkheteroaryl, aza, amide, ammonium, oxa, thia, sulfonyl, sulfinyl, sulfonamide, phosphoryl, phosphinyl, phosphino, phosphonium, keto, ester, alcohol, carbamate, urea, thiocarbonyl, borates, boranes, boraza, silyl, siloxy, silaza and combinations thereof; and combinations thereof. Thus, the PACPeD's useful in the present invention can have any combinations of substituted or unsubstituted R groups, saturated, partially saturated or unsaturated cyclics, ring structures, nitrogen containing heterocycles, or straps as defined above.

X, Y and Z represent suitable ligands or charge-neutralizing anions which are derived from any monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof (for example benzoic acid or benzoate anion, phenol or phenoxide anion, alcohol or alkoxide anion). X, Y and Z are independently selected from the group consisting of

halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxy, hydroperoxy, alkylperoxy, arylperoxy, ammonia, alkylamino, arylamino, heterocycloalkyl amino, heterocycloaryl amino, amine oxides, hydrazine, alkyl

5 hydrazine, aryl hydrazine, nitric oxide, cyanide, cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl nitrile, aryl nitrile, alkyl isonitrile, aryl isonitrile, nitrate, nitrite, azido, alkyl sulfonic acid, aryl sulfonic acid, alkyl sulfoxide, aryl sulfoxide, alkyl

10 aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid, aryl thiol thiocarboxylic acid, alkyl carboxylic acid (such as acetic acid,

15 trifluoroacetic acid, oxalic acid), aryl carboxylic acid (such as benzoic acid, phthalic acid), urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea, alkyl aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite,

20 hydrosulfite, alkyl phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide, alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, alkyl phosphinic acid, aryl

25 phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid, phosphate, thiophosphate, phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate,

30 alkyl thiocarbamate aryl thiocarbamate, alkyl aryl thiocarbamate, alkyl dithiocarbamate, aryl dithiocarbamate, alkyl aryl dithiocarbamate, bicarbonate, carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite,

35 tetrahalomanganate, tetrafluoroborate, hexafluorophosphate, hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra

alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate, and anions of ion exchange resins. The preferred ligands from which X, Y and Z are selected  
 5 include halide, organic acid, nitrate and bicarbonate anions.

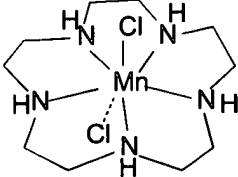
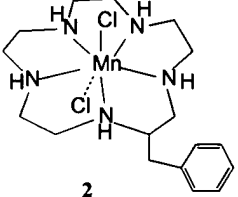
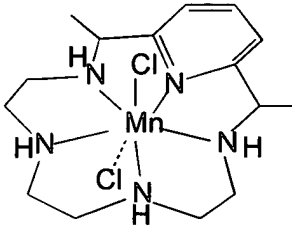
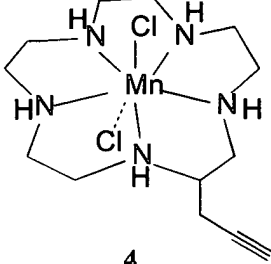
The "R" groups attached to the carbon atoms of the macrocycle can be in the axial or equatorial position relative to the macrocycle. When the "R" group is other  
 10 than hydrogen or when two adjacent "R" groups, i.e., on adjacent carbon atoms, together with the carbon atoms to which they are attached form a saturated, partially saturated or unsaturated cyclic or a nitrogen containing heterocycle, or when two R groups on the same carbon atom  
 15 together with the carbon atom to which they are attached form a saturated, partially saturated or unsaturated ring structure, it is preferred that at least some of the "R" groups are in the equatorial position for reasons of improved activity and stability. This is particularly  
 20 true when the complex contains more than one "R" group which is not hydrogen.

Where the modification of the biomaterial is effected by the surface covalent conjugation or copolymerization with the unmodified biomaterial, it is  
 25 preferred that the PACPeD contain a pendant reactive moiety. This reactive moiety may be on a "R" group, a cyclic, a heterocyclic, a nitrogen containing heterocyclic, or a strap structure as described above. Preferred moieties on the non-proteinaceous catalyst for  
 30 use in the present invention include of amino ( $-\text{NH}_2$ ), carboxyl ( $-\text{COOH}$ ), isocyanate ( $-\text{NCO}$ ), mercapto ( $-\text{SH}$ ), hydroxy ( $-\text{OH}$ ), silyl chloride ( $-\text{SiCl}_2$ ), acid halide ( $-\text{OCX}$  wherein  $\text{X} = \text{Cl}, \text{F}, \text{Br}, \text{I}$ ), halide ( $-\text{X}$  wherein  $\text{X} = \text{Cl}, \text{F}, \text{Br}, \text{I}$ ), glycidyl ( $-\text{HCOCH}_2$ ), and substituted or  
 35 unsubstituted alkenyl, alkynyl, and aryl moieties.

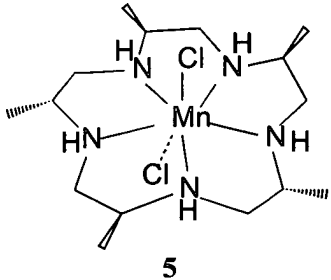
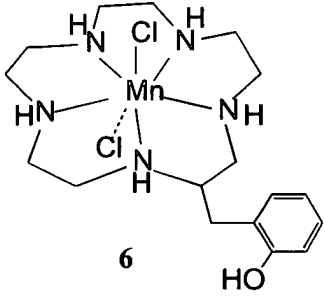
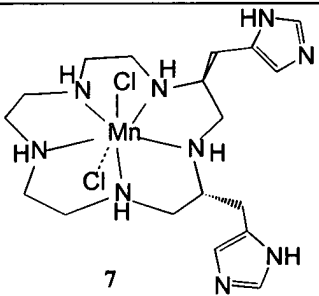
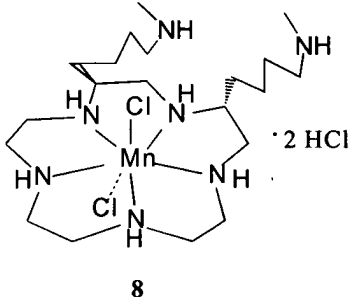
Preferred PACPeD's for modification of biomaterials compounds are those wherein at least one "R" group

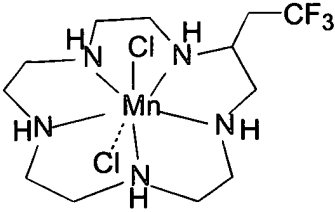
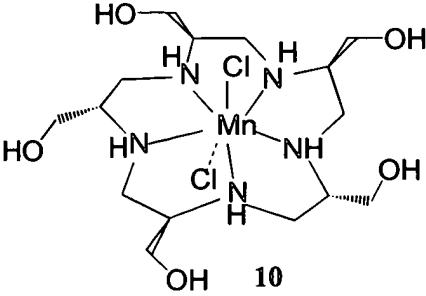
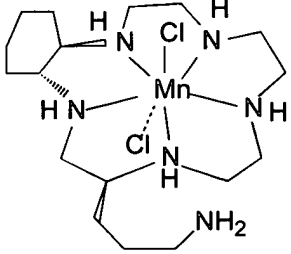
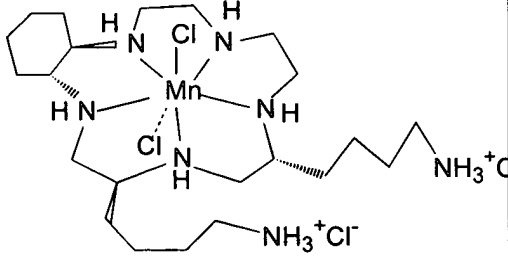
contains a reactive functional group, and those wherein at least one, of R or R' and R<sub>1</sub> or R'<sub>1</sub>, R<sub>2</sub> or R'<sub>2</sub> and R<sub>3</sub> or R'<sub>3</sub>, R<sub>4</sub> or R'<sub>4</sub> and R<sub>5</sub> or R'<sub>5</sub>, R<sub>6</sub> or R'<sub>6</sub> and R<sub>7</sub> or R'<sub>7</sub>, and R<sub>8</sub> or R'<sub>8</sub> and R<sub>9</sub> or R'<sub>9</sub>, together with the carbon atoms to  
5 which they are attached are bound to form a nitrogen containing heterocycle having 2 to 20 carbon atoms and all the remaining "R" groups are independently selected from hydrogen, saturated, partially saturated or unsaturated cyclic or alkyl groups. Examples of PACPeD  
10 catalysts useful in making the modified biomaterials of the invention include, but are not limited to, the following compounds:

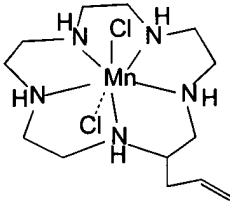
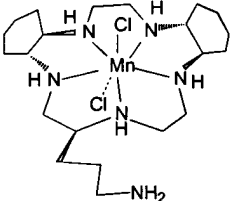
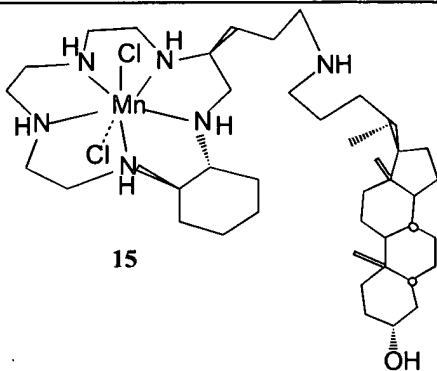
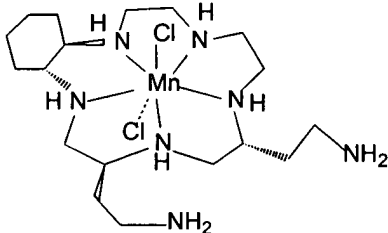
TABLE 1

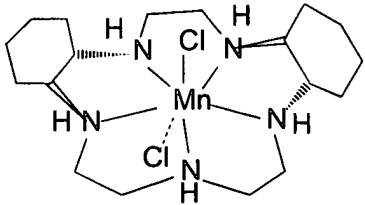
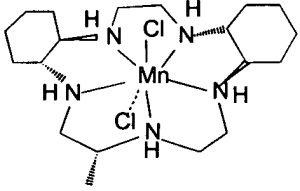
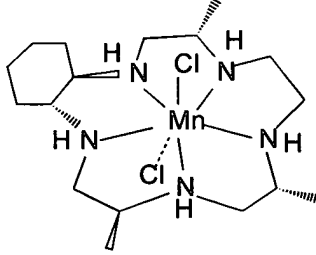
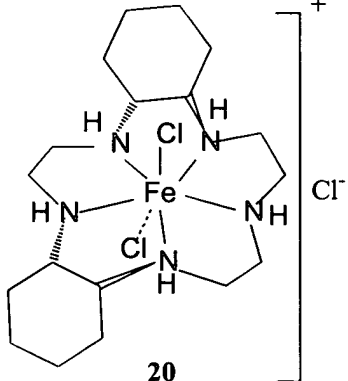
COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 <p>1</p>	341.19	4.13	2.24
 <p>2</p>	431.31	7.21	2.57
 <p>3</p>	403.26		1.00
 <p>4</p>	379.23		1.75



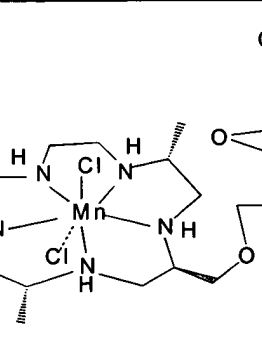
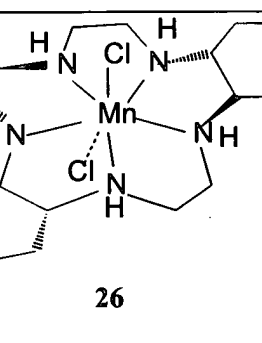
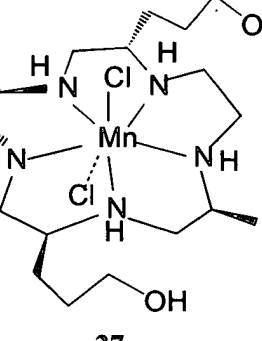
COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 5	411.77	3.82	3.90
 6	447.31	6.99	3.83
 7	501.37	2.00	1.58
 8	584.39	5.95	5.90

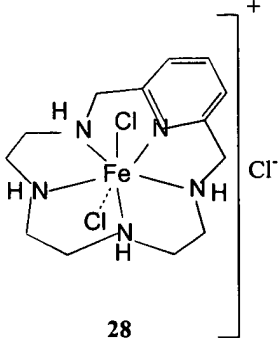
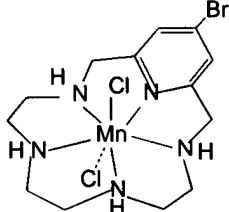
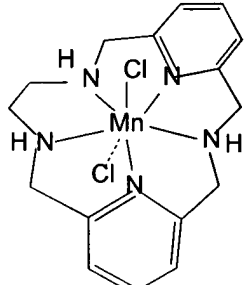
COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 <p>9</p>	423.22	2.77	1.68
 <p>10</p>	491.32	2.68	2.68
 <p>11</p>	452.37	4.79	2.85
 <p>12</p>	610.42	10.20	5.39

COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 <p>13</p>	383.27		1.63
 <p>14</p>	506.46	7.58	3.84
 <p>15</p>	795.95	2.41	0.77
 <p>16</p>	481.41	2.48	1.97

COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 17	449.37	12.60	4.09
 18	463.40	15.00	4.00
 19	437.36	8.48	4.08
 20	485.70	3.29	0.93

COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
	599.67	2.93	1.29
22	494.63	11.40	5.03
	461.29	6.61	3.47
23			
	493.38	2.55	2.55
24			

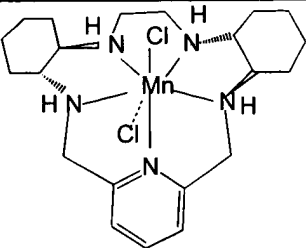
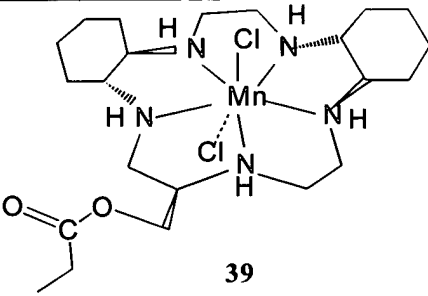
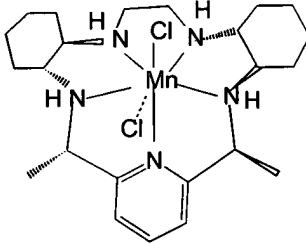
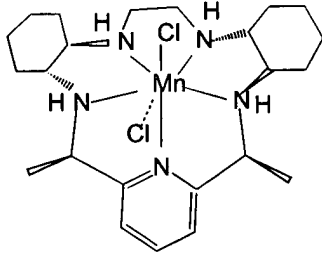
COMPOUND	MOL. WT.	$k_{cat}$ pH 7.4	$k_{cat}$ pH 8.1
 <p>25</p>	724.39	4.04	2.34
 <p>26</p>	479.40	10.12	3.47
 <p>27</p>	525.47	4.83	2.50

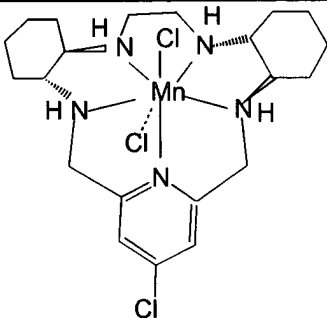
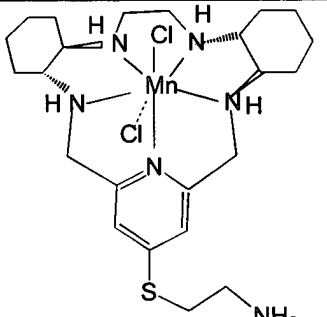
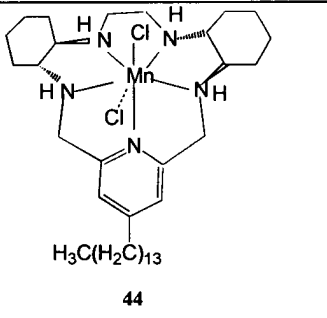
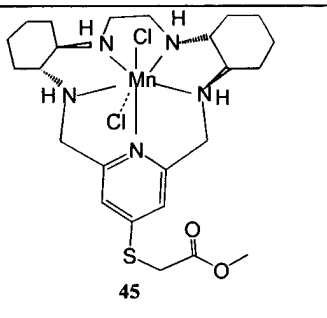
COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 28	411.56		
 29	454.10	2.86	2.02
 30	409.22	0.20	0.20

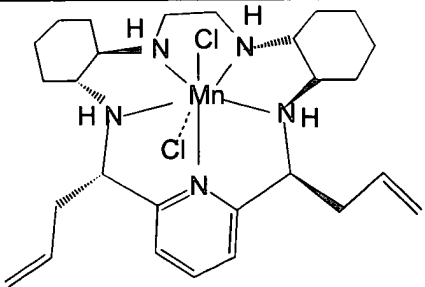
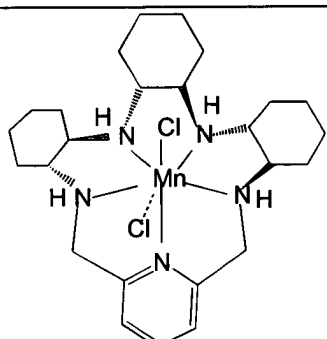
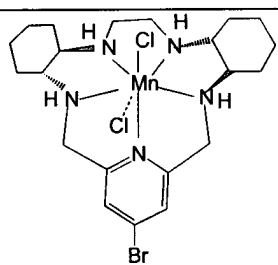


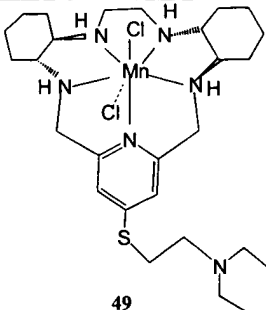
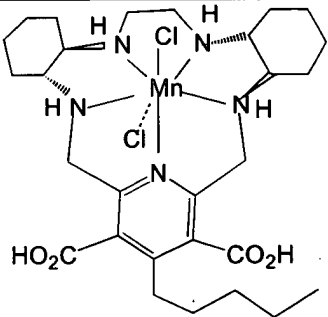
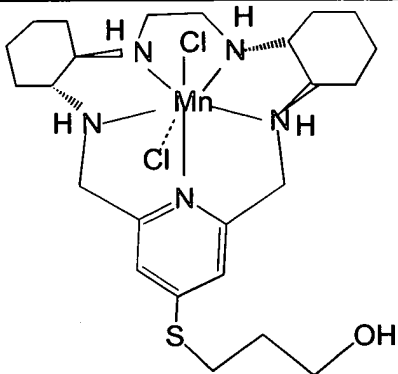


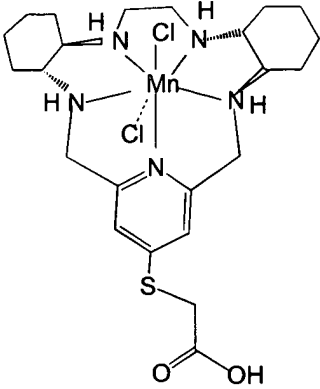
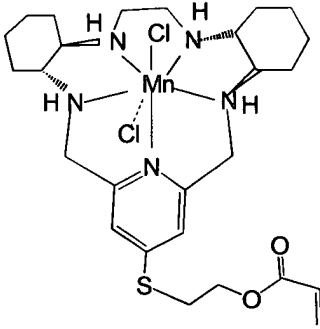
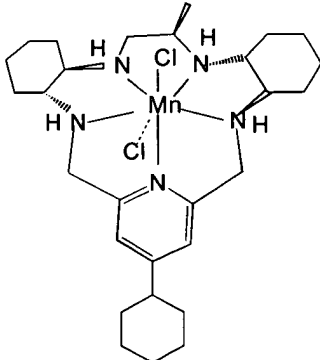


COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 <p>38</p>	483.39	1.64	1.19
 <p>39</p>	535.46	3.89	2.32
 <p>40</p>	511.44	90.00	11.00
 <p>41</p>	511.44	1.57	0.41

COMPOUND	MOL. WT.	k <sub>cat</sub> pH 7.4	k <sub>cat</sub> pH 8.1
 <b>42</b>	517.83	1.18	0.98
 <b>43</b>			
 <b>44</b>	679.76	1.02	0.84
 <b>45</b>	587.51	2.99	0.95

COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 46	563.52		
 47	537.48		2.16
 48	562.28	1.68	

COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 49	614.52		
 50	641.50	1.31	
 51	573.53	3.97	1.14

COMPOUND	MOL. WT.	$k_{\text{cat}}$ pH 7.4	$k_{\text{cat}}$ pH 8.1
 52	537.02	3.01	
 53			
 54	579.56	2.68	

Activity of the non-proteinaceous catalysts for the dismutation of superoxide can be demonstrated using the stopped-flow kinetic analysis technique as described in Example 24, and in Riley, D. P., Rivers, W. J. and Weiss, R. H., "Stopped-Flow Kinetic Analysis for Monitoring Superoxide Decay in Aqueous Systems," Anal. Biochem., 196, 344-349 (1991), which is incorporated by reference herein. Stopped-flow kinetic analysis is an accurate and direct method for quantitatively monitoring the decay rates of superoxide in water. The stopped-flow kinetic analysis is suitable for screening compounds for SOD activity and activity of the compounds or complexes of the present invention, as shown by stopped-flow analysis, correlate to usefulness in the modified biomaterials and processes of the present invention. The catalytic constants given for the exemplary compounds in the table above were determined using this method.

As can be observed from the table, a wide variety of PACPeD's with superoxide dismutating activity may be readily synthesized. Generally, the transition metal center of the catalyst is thought to be the active site of catalysis, wherein the manganese or iron ion cycles between the (II) and (III) states. Thus, as long as the redox potential of the ion is in a range in which superoxide anion can reduce the oxidized metal and protonated superoxide can oxidize the reduced metal, and steric hindrance of the approach of the superoxide anion is minimal, the catalyst will function with a  $k_{cat}$  of about  $10^6$  to  $10^8$ .

Without limiting themselves to any particular theory, applicants propose that the mechanism described in Riley, et al., 1999, is a reasonable approximation of how the PACPeD catalysts dismutate superoxide. In order

for the complex to exhibit superoxide dismutase activity, the ligand should be able to fold into a conformation that allows the stabilization of an octahedral complex between the superoxide anion and the five nitrogens of the ligand ring. If a compound contains several  
5 conjugated double bonds within the main 15-membered ring of the ligand, which hold the ring in a rigid conformation, the compound would not be expected to exhibit catalytic activity. R groups which are  
10 coordinated with the transition metal ion freeze the conformation of the ligand, and would be expected to be poor catalysts. Large, highly electronegative groups pendant on the macrocycle would also sterically hinder the necessary conformational change. The lack of  
15 functionality in these types of PACPeD derivatives would not be unexpected by one of ordinary skill in the art. Specifically, one of skill in the art would avoid materially changing the flexibility of the PACPeD by adding many large groups which would cause steric  
20 hindrance, or placing too many double bonds into the main PACPeD ring. This effect would also be present in certain geometric arrangements of smaller R groups which constrain the complex to a rigid, planar geometry. Those particular compounds which do not exhibit superoxide  
25 dismutase activity should not be used to modify the biomaterials of the present invention.

Given these examples and guidelines, one of ordinary skill would be able to choose a PACPeD catalyst for use  
30 in the present invention which would contain any required functional group, while still retaining superoxide dismutating activity. The PACPeD catalysts described above may be produced by the methods disclosed in U.S. Pat. No. 5,610,293. However, it is preferred that the  
35 PACPeD catalysts used in the present invention be synthesized by the template method, diagramed below. This synthesis method is advantageous over previously



disclosed methods in that cyclization yields utilizing the template method are usually about 90%, as compared to about 20% with previous methods. Several diamines are commercially available as starting materials, or a

5 diamine may be synthesized. The diamine is reacted with titryl chloride in anhydrous methylene chloride at 0°C and allowed to warm to room temperature overnight, with stirring. The product is then combined with glyoxal in methanol and stirred for 16 hours. The glyoxal bisimine

10 product is then reduced with a borohydride in THF. If a non-symmetrical product is desired, two diamines may be used as starting materials. In addition, a substituted glyoxal may be used if groups pendant from the macrocycle opposite the pyridine are desired ( $R_5$  and  $R_4$ ).

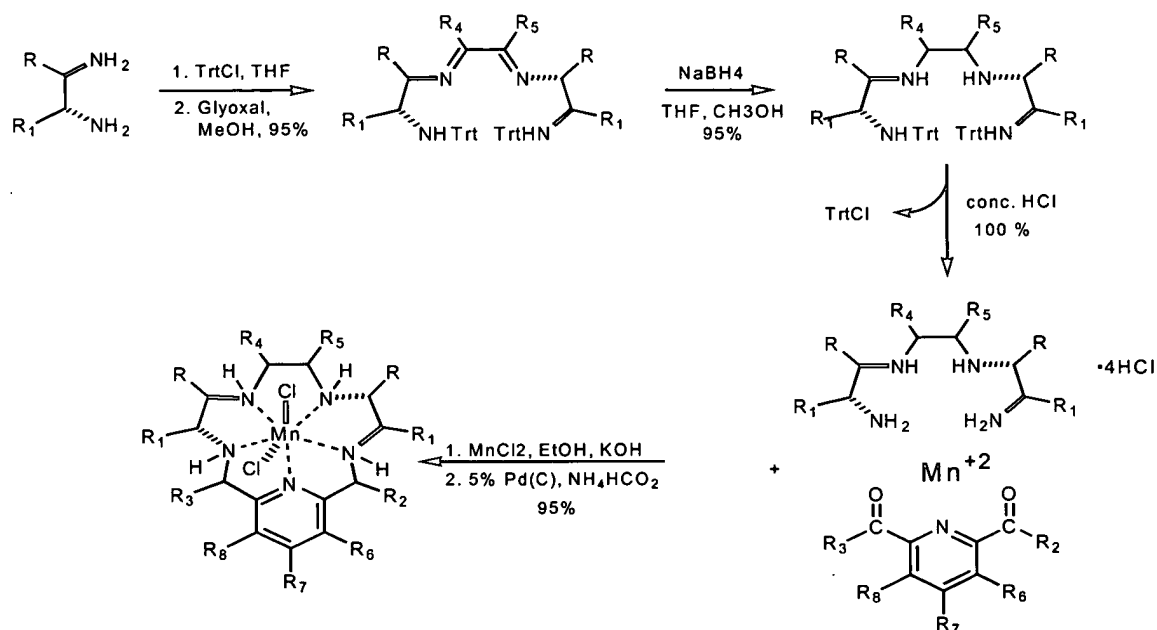
15 Commercially available tetraamines may also be used in place of the reduced glyoxal bisimine. After reduction of the glyoxal bisimine, the product is combined with a 2,6 dicarbonyl substituted pyridine, such as 2,6, dicarboxaldehyde pyridine or 2,6 diacetyl pyridine, and

20 a salt of manganese or iron under basic conditions. The transition metal ion serves as a template to promote cyclization of the substituted pyridine and the tetraamine. Several 2,6 dicarbonyl substituted pyridines are available commercially, allowing for the facile

25 production of a variety of ligands with groups pendant from the macrocycle proximal to the pyridine ( $R_2$  and  $R_3$ ). Additionally, pyridines with additional substitutions ( $R_6$ ,  $R_7$  and  $R_8$ ) may also be used. After cyclization, the product is reduced with ammonium formate and a palladium

30 catalyst over a period of 3-4 days. In addition to the "R" substitutions, "R'" groups may also be substituted at the same carbons. "R" and "R'" groups may be any of those indicated above. The process may be varied according to principles well known to one of ordinary

35 skill in the art in order to accommodate various starting materials.



Although the bisimine produced in the template cyclization reaction step above may be reduced by more conventional means using hydrogen gas, it is preferred that the bisimine be reduced with ammonium formate in the presence of a palladium catalyst, as illustrated in Example 6. This process offers the advantages of increased safety and high reduction efficiency.

The PACPeD's useful in the present invention can possess one or more asymmetric carbon atoms and are thus capable of existing in the form of optical isomers as well as in the form of racemic or nonracemic mixtures thereof. The optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes, for example by formation of diastereoisomeric salts by treatment with an optically active acid. Examples of appropriate acids are tartaric, diacetyltartaric, dibenzoyltartaric, ditoluoyltartaric and camphorsulfonic acid and then separation of the mixture of diastereoisomers by crystallization followed by liberation of the optically active bases from these salts. A different process for separation of optical

15           Also suitable for use in the present invention, but  
less preferred than the PACPeD's, are the salen complexes  
of manganese and iron disclosed in U.S. Patent No.  
5,696,109, here incorporated by reference. The term  
"salen complex" means a ligand complex with the general  
20 formula:



wherein M is a transition metal ion, preferably Mn; A is an anion, typically Cl; and n is either 0, 1, or 2. X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub> and X<sub>4</sub> are independently selected from the group consisting of hydrogen, silyls, aryls, aryls, arylalkyls, primary alkyls, secondary alkyls, tertiary alkyls, alkoxys, aryloxys, aminos, quaternary amines, heteroatoms, and hydrogen; typically X<sub>1</sub> and X<sub>3</sub> are from the same functional group, usually hydrogen, quaternary amine, or tertiary butyl, and X<sub>2</sub> and X<sub>4</sub> are typically hydrogen. Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>3</sub>, Y<sub>4</sub>, Y<sub>5</sub>, and Y<sub>6</sub> are independently selected from the group consisting of hydrogen, halides,

alkyls, aryls, arylalkyls, silyl groups, aminos, alkyls or aryls bearing heteroatoms; aryloxys, alkoxys, and halide; preferably,  $Y_1$  and  $Y_4$  are alkoxy, halide, or amino groups. Typically,  $Y_1$  and  $Y_4$  are the same.  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently selected from the group consisting of H,  $CH_3$ ,  $C_2H_5$ ,  $C_6H_5$ , O-benzyl, primary alkyls, fatty acid esters, substituted alkoxyaryls, heteroatom-bearing aromatic groups, arylalkyls, secondary alkyls, and tertiary alkyls. Methods of synthesizing these salen complexes are also disclosed in U.S. Patent No. 5,696,109.

Iron or manganese porphyrins, such as, such as  $Mn^{III}$  tetrakis(4-N-methylpyridyl)porphyrin,  $Mn^{III}$  tetrakis-o-(4-N-methylisonicotinamidophenyl)porphyrin,  $Mn^{III}$  tetrakis(4-N-N-N-trimethylanilinium)porphyrin,  $Mn^{III}$  tetrakis(1-methyl-4-pyridyl)porphyrin,  $Mn^{III}$  tetrakis(4-benzoic acid)porphyrin,  $Mn^{II}$  octabromo-meso-tetrakis(N-methylpyridinium-4-yl)porphyrin,  $Fe^{III}$  tetrakis(4-N-methylpyridyl)porphyrin, and  $Fe^{III}$  tetrakis-o-(4-N-methylisonicotinamidophenyl)porphyrin. may also be used in the present invention. The catalytic activities and methods of purifying or synthesizing these porphyrins are well known in the organic chemistry arts.

The salen and porphyrin non-proteinaceous catalysts for the dismutation of superoxide also preferably contain a reactive moiety, as described above, when the methods of surface covalent conjugation or copolymerization are used to modify the biomaterial.

In general, the non-proteinaceous catalysts for the dismutation of superoxide used in the present invention are very stable under conditions of high heat, acid or basic conditions, and in a wide variety of solvents. However, under extreme reaction conditions the chelated transition metal ion will dissociate from the non-proteinaceous catalyst. Thus, when extreme reaction conditions are necessary to modify the biomaterial, it is preferable to modify the biomaterial with a precursor

ligand of the non-proteinaceous catalyst for the  
dismutation of superoxide, and then afterwards react the  
modified biomaterial with a compound containing the  
appropriate transition metal in order to produce a  
5 biomaterial modified with an active non-proteinaceous  
catalyst for the dismutation of superoxide. For  
instance, when a PACPeD catalyst is used under reaction  
conditions of  $\text{pH} < 4$ , the strategy of modifying the  
biomaterial with the ligand should be used. This strategy  
10 is demonstrated in Example 19. Therefore, when the term  
non-proteinaceous catalyst for the dismutation of  
superoxide is used in this specification, the reader  
should assume that, where appropriate, the precursor  
ligand will be used in the modification of the  
15 biomaterial, and that the transition metal cation  
necessary for activity may be added at a later point in  
time. Conditions where this approach would be  
appropriate may be readily determined by one of ordinary  
skill in the chemical arts.

20

#### Choice of Method of Modification

As previously described, the biomaterials of the  
present invention may be modified by the diverse methods  
of surface covalent conjugation, copolymerization, or  
25 admixture. The methods of surface covalent conjugation  
and copolymerization use covalent bonds in order to  
physically associate the non-proteinaceous catalyst for  
the dismutation of superoxide with the biomaterial. This  
creates a very stable physical association which  
30 preserves the superoxide dismutating activity of the  
modified biomaterial. In contrast, non-covalent forces  
create the physical association between the biomaterial  
and the non-proteinaceous catalysts for the dismutation  
of superoxide when the technique of physical admixture is  
35 used. These non-covalent forces may be weak Van der Waal's  
forces, or they may be stronger ionic bonding or  
hydrophobic interaction forces. Although ionic or

hydrophobic interactions between the non-proteinaceous catalyst and the biomaterial will prevent elution of the non-proteinaceous catalyst to some degree, the catalyst will still be lost from the biomaterial over time when  
5 the biomaterial is exposed to biological tissues or fluids. Thus, it is usually preferred that the methods of covalent surface conjugation or copolymerization be used to modify biomaterials which will be exposed to biological systems for prolonged periods of time.  
10 However, uses may arise where the elution of non-proteinaceous catalysts for the dismutation of superoxide into the tissues surrounding an article comprising the modified biomaterial may be desirable. In this case, the use of biomaterials modified by the physical admixture  
15 method would be appropriate.

When composite materials are used, it may be necessary to utilize a variety of modification techniques. For instance, in a biomaterial composed of hydroxyapatite and polyethylene, a non-proteinaceous  
20 catalyst may be admixed with the hydroxyapatite phase of the composite, and another copolymerized with the polyethylene phase of the composites. The two composites may then be joined together into a fully modified composite biomaterial. Similarly, a composite material  
25 which utilizes carbon fiber and polypropylene could be made using a copolymerized polypropylene and a surface covalently conjugated carbon fiber. The flexibility in the production of modified biomaterials offered by the processes of the invention allows for the use of several  
30 diverse materials in a device while increasing its durability and decreasing the inflammatory response to the device.

Generally, it is preferred that the non-proteinaceous catalyst be present in an amount of about  
35 0.001 to 25 weight percent. It is more preferable that the catalyst be present in an amount of about 0.01 to 10 weight percent. It is most preferable that the catalyst

be present in an amount of about 0.05 to 5 weight percent. However, the amount of the non-proteinaceous catalyst to be used in modifying the biomaterial will depend on several factors, including the characteristics of the catalyst, the characteristics of the biomaterial, and the method of modification used. As is evident from the chart above, the catalytic activity of the non-proteinaceous catalysts for use in the present invention may vary over several orders of magnitude. Thus, less of the more efficient catalysts will be needed to obtain the same protective effects. Also, some biomaterials are more inflammatory than others. Thus, a greater amount of catalyst should be used with these biomaterials in order to counteract the strong inflammatory foreign body response that they provoke. In addition, the amount of catalyst used to modify the biomaterial should not be so high as to significantly alter the mechanical characteristics of the biomaterial. Because a covalently conjugated catalyst is concentrated at the surface of the biomaterial used in a device, almost all of the catalyst will interact with the biological environment. Conversely, because an admixed or copolymerized catalyst is dispersed throughout the biomaterial, less of the catalyst will be available to interact with the biological environment at the surface of the biomaterial. Thus, when the catalyst is covalently conjugated to the surface of the biomaterial, less catalyst will be needed than if the catalyst is admixed or copolymerized with the biomaterial. Given the above considerations, the person of ordinary skill in the art would be able to choose a proper amount of non-proteinaceous catalyst to use in the present invention in order to achieve the desired reduction in the inflammatory response and degradation.

It is to be understood that although the non-proteinaceous catalysts used in the following processes are usually referred to in the singular, multiple catalysts may be used in any of these processes. One of

ordinary skill in the art will easily be able to choose complementary catalysts for such modified biomaterials. In addition, although not specifically enumerated herein, the combination of the biomaterial modification techniques of the present invention with other biomaterial modification techniques, such as heparin coating, is contemplated within the present invention.

#### Modification by Surface Covalent Conjugation

- 10       The general process for producing a biomaterial modified by surface covalent conjugation with at least one non-proteinaceous catalyst for the dismutation of superoxide or at least one precursor ligand of a non-proteinaceous catalyst for the dismutation of superoxide, comprises:
- 15           a.               providing at least one reactive functional group on a surface of the biomaterial to be modified;
- 20           b.               providing at least one complementary reactive functional group on the non-proteinaceous catalyst for the dismutation of superoxide or on the precursor ligand; and
- 25           c.               conjugating the non-proteinaceous catalyst for the dismutation of superoxide or the precursor ligand with the surface of the biomaterial through at least one covalent bond.

      This process may be effected by a photo-chemical reaction, or any of a number of conjugating reactions known in the art, such as condensation, esterification, oxidative, exchange, or substitution reactions. Preferred conjugation reactions for use in the present invention do not involve extreme reaction conditions, such as a temperature above about 375EC, or pH less than about 4. In addition, it is preferred that the conjugation reaction not produce a covalent bond that is readily cleaved by common enzymes found in biological



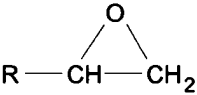
systems. Usually, it is desirable for the non-proteinaceous catalyst to have only one complementary functional group. However, in cases where crosslinking of the biomaterial is desired, such as in hydrogels, 5 poly-functional-group catalysts may be used. Care should be taken, however, to choose functional groups which will not allow the non-proteinaceous catalyst to self-polymerize, as this will decrease the efficiency of the conjugation reaction. Likewise, multiple non-10 proteinaceous catalysts may be used to modify the biomaterial, although complementary functional groups which allow avoid inter-catalyst conjugations would not be preferred.

The non-proteinaceous catalyst for the dismutation 15 of superoxide or the precursor ligand may be covalently bound directly to the surface of the biomaterial, or bound to the surface through a linker molecule. Where the non-proteinaceous catalyst and the surface of the biomaterial are directly conjugated, the reactive 20 functional group and the complementary reactive functional group will form a covalent bond in the conjugation reaction. For instance, poly(ethyleneterephthalate) may be hydrolyzed to carboxyl functional groups. Compound 43 may then be reacted with 25 the derivatized polymer to form the amide bond, as illustrated in Example 7. Examples H and E also illustrate a direct surface covalent conjugation. Further suggestions for reactive groups to use in of direct conjugation may be found in U.S. Pat. No. 30 5,830,539, herein incorporated by reference. Several exemplary paired functional groups are given in Table 2:

TABLE 2

Non-proteinaceous Catalyst (SODm) Group	Substrate (R) Group	Resulting Linkage
SODm—NH <sub>2</sub>	$R-N=C=O$	$\begin{array}{c} \text{H} \quad \text{O} \quad \text{H} \\   \quad    \quad   \\ R-N-C-N-SODm \end{array}$
SODm—NH <sub>2</sub>	$\begin{array}{c} \text{O} \\    \\ R-C-OH \end{array}$	$\begin{array}{c} \text{O} \quad \text{H} \\    \quad   \\ R-C-N-SODm \end{array}$
SODm—NH <sub>2</sub>	$\begin{array}{c} \text{O} \\    \\ R-C-X \end{array}$ <p>(X=Cl, F, Br, I)</p>	$\begin{array}{c} \text{O} \quad \text{H} \\    \quad   \\ R-C-N-SODm \end{array}$
SODm—NH <sub>2</sub>	$\begin{array}{c} \text{O} \\    \\ R-C-H \end{array}$	$R-CH=NH-SODm$
SODm—NH <sub>2</sub>	$\begin{array}{c} \text{O} \\ \diagup \quad \diagdown \\ R-CH \quad CH_2 \end{array}$	$\begin{array}{c} \text{H} \\   \\ R-CH-CH_2-N-SODm \\   \\ OH \end{array}$

SODm—NH <sub>2</sub>	R—N=C=S	$\begin{array}{c} \text{H} \quad \text{S} \quad \text{H} \\   \quad    \quad   \\ \text{R}-\text{N}-\text{C}-\text{N}-\text{SODm} \end{array}$
SODm—OH	R—N=C=O	$\begin{array}{c} \text{H} \quad \text{O} \\   \quad    \\ \text{R}-\text{N}-\text{C}-\text{O}-\text{SODm} \end{array}$
SODm—OH	$\begin{array}{c} \text{O} \\    \\ \text{R}-\text{C}-\text{OH} \end{array}$	$\begin{array}{c} \text{O} \\    \\ \text{R}-\text{C}-\text{O}-\text{SODm} \end{array}$
SODm—OH	$\begin{array}{c} \text{O} \\ \diagup \quad \diagdown \\ \text{R}-\text{CH}-\text{CH}_2 \end{array}$	$\begin{array}{c} \text{OH} \\   \\ \text{R}-\text{CH}-\text{CH}_2-\text{O}-\text{SODm} \end{array}$
SODm—OH	R—N=C=S	$\begin{array}{c} \text{H} \quad \text{S} \\   \quad    \\ \text{R}-\text{N}-\text{C}-\text{O}-\text{SODm} \end{array}$
SODm—OH	$\begin{array}{c} \text{O} \\    \\ \text{R}-\text{C}-\text{X} \end{array}$	$\begin{array}{c} \text{O} \\    \\ \text{R}-\text{C}-\text{O}-\text{SODm} \end{array}$
SODm—OH	R—Si—(OCH <sub>3</sub> ) <sub>3</sub>	R—Si—(O—SODm) <sub>3</sub>
$\begin{array}{c} \text{O} \\    \\ \text{SODm}-\text{C}-\text{OH} \end{array}$	R—OH	$\begin{array}{c} \text{O} \\    \\ \text{R}-\text{O}-\text{C}-\text{SODm} \end{array}$

$\text{SODm}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{OH}$	$\text{R}-\text{N}=\text{C}=\text{O}$	$\text{R}-\overset{\overset{\text{H}}{\mid}}{\text{N}}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{O}-\text{SODm}$
$\text{SODm}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{OH}$	$\text{R}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{X}$	$\text{R}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{O}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{SODm}$
$\text{SODm}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{OH}$	$\text{R}-\text{CH}-\text{CH}_2$ 	$\text{R}-\overset{\overset{\text{OH}}{\mid}}{\text{CH}}-\text{CH}_2-\text{O}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{SODm}$
$\text{SODm}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{OH}$	$\text{R}-\text{NH}_2$	$\text{R}-\overset{\overset{\text{H}}{\mid}}{\text{N}}-\overset{\overset{\text{O}}{\parallel}}{\text{C}}-\text{O}-\text{SODm}$

When a linker molecule is used, the above process further comprises providing at least one linker capable of reacting with both the reactive functional group on a surface of the biomaterial to be modified and the complementary reactive functional group on the non-proteinaceous catalyst for the dismutation of superoxide or the precursor ligand. During the conjugation process, the reactive functional group on the surface of the article and the complementary reactive functional group on the non-proteinaceous catalyst for the dismutation of superoxide form a covalent bond with the linker. This process may occur all in one step, or in a series of steps. For instance, in a two step process, a carboxyl functionalized polymer, such as a hydrolyzed poly(ethyleneterephthalate) polymer ("PET") could first

be reacted with a (Gly)<sub>12</sub> linker in an amide reaction. Then, after removal of excess linker, the PET- glycine linker could react with an amino PACPeD such as Compound 43 to form a polymer-glycine linker-Compound 43 modified  
5 biomaterial. Alternately, the hydrolyzed PET could be linked with a low molecular weight PEG to a carboxyl PACPeD such as Compound 52 by an ester reaction in a single step. Linkers suitable for use in this process include polysaccharides, polyalkylene glycols,  
10 polypeptides, polyaldehydes, and silyl groups. Silyl groups are particularly useful in conjugating non-proteinaceous catalysts with metal biomaterials. Examples of linkers and functional groups which are useful in the present invention may be found in U.S. Pat.  
15 Nos. 5,877,263 and 5,861,032. Persons of ordinary skill in the chemical arts will be able to determine an appropriate linker and non-proteinaceous catalyst for conjugation to any biomaterial, including metals, ceramics, polymers, biopolymers, and various phases of  
20 composites.

This method of modification may be used with an article which is already in its final form, or may be used with parts of an article before final assembly. In addition, this method is useful for modifying thin stock  
25 materials which will be used in the later manufacture of a device, such as polymer or chitosan films, or fibers which will be woven into fabrics for vascular grafts. This method is also useful for modifying diverse materials in a single step with one non-proteinaceous  
30 catalyst. For instance, a tantalum component which has been reacted with a silyl linker, as in Example 13, and a poly(ethyleneterephthalate) component which has been hydrolyzed, as in Example 7, may be assembled into a final device. Then, Compound 43 could be reacted with  
35 the entire article to modify the surface of both materials in a single step.

### Modification by Copolymerization

Biomaterials may also be modified according to the present invention by co-polymerization with a non-proteinaceous catalyst for the dismutation of superoxide or the ligand precursor of a non-proteinaceous catalyst for the dismutation of superoxide. This process, in general, comprises:

- a. providing at least one monomer;
- b. providing at least one non-proteinaceous catalyst for the dismutation of superoxide or at least one ligand precursor of a non-proteinaceous catalyst for the dismutation of superoxide containing at least one functional group capable of reaction with the monomer and also containing at least one functional group capable of propagation of the polymerization reaction,
- c. copolymerizing the monomers and the non-proteinaceous catalyst for the dismutation of superoxide or the ligand precursor in a polymerization reaction.

The copolymerization technique is advantageous for the modification of polymers and synthetic biopolymers with non-proteinaceous catalysts for the dismutation of superoxide. However, it is preferred that this method be used with polymers whose polymerization reaction occurs at temperatures less than about 37°C, and pH greater than about 4. If the polymerization reaction is carried out at a pH less than 4, a ligand precursor of the non-proteinaceous catalysts for the dismutation of superoxide should be used. Monomers useful in this process include alkynes, vinyls, vinyl halides, vinylidenes, diacids, acid amines, diols, alcohol acids, alcohol amines, diamines, ureas, urethanes, phthalates, carbonic acids, orthoesters, esteramines, siloxanes, phosphazenes, olefins, alkylene halides, alkylene oxides, acrylic

acids, sulfones, anhydrides, acrylonitriles, saccharides, and amino acids.

As demonstrated previously, the non-proteinaceous catalysts for the dismutation of superoxide used in the present invention may be synthesized with any functional group necessary to react with the any of these monomers. In order to prevent the termination of the polymerization reaction, it is necessary that the non-proteinaceous catalyst also contain a polymerization propagation functional group. Often, this will be another functional group identical to the first functional group, as in the diamine PACPeD Compound 16. This catalyst is copolymerized with polyureaurethane in Example 16. However, as when the polymerization reaction involves a vinyl reaction, the reactive and propagative functional groups may be the same, such as in the acryloyl derivatized Compound 53. Copolymerization of this catalyst with acrylic or methacrylic is shown in Example 17. Example 18 also illustrates the modification of biomaterials by copolymerization with non-proteinaceous catalysts.

Biomaterials modified by copolymerization have several advantages. First, the non-proteinaceous catalysts for the dismutation of superoxide are covalently bound to the modified biomaterial, preventing dissociation of the catalysts and a loss of function. Second, the modification of the material is continuous throughout the biomaterial, allowing for continuous protection by the catalyst if the exterior surface of the material is by mechanical or chemical degradation. Third, the material can be melted and re-formed into any useful article after modification, provided that the polymer melts below about 375EC. Alternatively, wet-spinning or solvent casting may be used to make articles from these modified polymer biomaterials. These characteristics make the modified polymer biomaterials

produced by this process a versatile tool for various medical device applications.

#### Modification by Admixture

- 5           The biomaterials of the present invention may also be modified by admixture with at least one non-proteinaceous catalyst for the dismutation of superoxide or a precursor ligand of a non-proteinaceous catalyst for the dismutation of superoxide. The general process
- 10 comprises:
- a.           providing at least one unmodified biomaterial;
  - b.           providing at least one non-proteinaceous catalyst for the dismutation of superoxide or at least one ligand precursor of
  - 15 a non-proteinaceous catalyst for the dismutation of superoxide; and
  - c.           admixing the unmodified biomaterial and the non-proteinaceous catalyst
  - 20 for the dismutation of superoxide or the ligand precursor.

Biomaterials modified according to this process preferably form a solution with the non-proteinaceous catalyst or ligand, although a  $\mu$ m to nm-sized particle mixture is also contemplated by the present invention. The above admixture process may involve heating the constituents in order to melt at least one unmodified biomaterial constituent. For instance, the PACPeD

30 catalyst Compound 38 can be mixed with melted polypropylene at 250EC, as in Example 20. Many other polymer biomaterials melt below 300EC, such as polyethylene, poly(ethyleneterephthalate) and polyamides, and would be especially suitable for use in this melted

35 admixture technique. After admixing, the melted modified biomaterial may be injection or extrusion molded, or



spun. Temperatures above about 375EC should not be used, however, as decomposition of the catalyst may result.

Thus, metals, ceramics, and high-melt polymers should not be melted for admixture. Rather, a solvent in  
5 which at least one unmodified biomaterial and the non-proteinaceous catalyst for the dismutation of superoxide or the ligand precursor are soluble may be used when admixing these constituents. As noted above, the PACPeD catalysts are soluble in several common solvents. If the  
10 solvent method is used, the process preferably further comprises removing the solvent after admixing. Methods suitable for removing a solvent used in the present invention include evaporation and membrane filtration, although care should be taken so that the membrane filter  
15 size will retain the non-proteinaceous catalyst. As with the copolymerized modified biomaterials, the admixed modified biomaterials may be wet spun or solution cast.

More hydrophobic or hydrophilic groups may be added to the non-proteinaceous catalyst in order to change its  
20 solubility characteristics. Likewise, the non-proteinaceous catalysts may be synthesized with specific pendant groups in order to have a particular affinity for the modified biomaterial. Usually this is accomplished by choosing the non-proteinaceous catalyst used in the  
25 admixture process so that ionic or hydrophobic interactions will occur between the catalysts and the modified biomaterial. For instance, the negatively charged carboxyl group of Compound 52 would have an affinity for the positively charged calcium ions in a  
30 hydroxyapatite ceramic matrix. Similarly, the added cyclohexyl group of Compound 47, as well as the lack of pendant polar groups, would help this catalyst to integrate into polyethylene. Thus, by increasing the affinity of the non-proteinaceous catalyst for the  
35 biomaterial, one can help to prevent the dissociation of the catalyst from the modified biomaterial.

### Uses of the Modified Biomaterials

The biomaterials of the present invention show greatly improved durability and decreased inflammatory response when interacting with biological systems. Thus, these biomaterials modified with non-proteinaceous catalysts for the dismutation of superoxide are ideal for use in devices for implantation or the handling of bodily fluids. Since the non-proteinaceous catalysts for the dismutation of superoxide are not consumed during the dismutation reaction, they may retain their activity indefinitely. The biocompatible article can be an article where, during its intended use, at least a portion of the article comprising the modified biomaterial is implanted within a mammal. For instance, one such application would be coating pacemaker lead wires as described in U.S. Pat. No. 5,851,227 with the modified polyureaurethane of Example 16. These improved lead wires are believed to be more durable in the body, and thus prevent the device failure which is often seen with conventional polyurethane coated wires. Similarly, a modified polyester, such as in Example 19, could be used to spin fibers for vascular graft fabric as described in U.S. Pat. No. 5,824,047. Grafts made using this fabric are believed to heal faster, as less inflammation would be caused by the biomaterial. Similarly, the modified polypropylene tested in Example 22 could be used to make surgical sutures. The biocompatible article may also be one where, during its intended use, the surface comprising the modified biomaterial is exposed to biological fluids, such as blood or lymph. For instance, a surface covalently conjugated chitosan film would be ideal for use as a membrane material in heart-lung machines which oxygenate and circulate blood during bypass operations. The copolymerized poly(etherurethane urea) of Example 16 would be useful in manufacturing the direct mechanical bi-ventricular cardiac assist device of U.S. Pat. No. 5,749,839. Use of these biomaterials in

tissue engineering devices, such as scaffoldings, would be another application.

The various methods of modifying biomaterials provided by the invention allow for a wide range of practical applications. For instance, in manufacturing stents for use in angioplasty procedures, one would have the option of directly conjugating a PACPeD with a pendant silyl group with the steel of a stent manufactured as described in U.S. Pat. No. 5,800,456, through the formation of a covalent bond. Alternatively, one could copolymerize a PACPeD with pendant amine groups with a polyurethane, as in Example 16, and coat the stent with the polymer. Yet another option would be to admix a PACPeD with polypropylene, as in Example 20, extrude the mixture into a stretchable film, and shrink wrap the stent in the modified polymer film. As shown by this simple example, the diverse processes for the production of modified biomaterials using non-proteinaceous catalysts for the dismutation of superoxide allow the bio-engineer a wide variety of manufacturing techniques. A person of ordinary skill in the art of medical device design would be able to discern which modified material, and which process of modification, would be best for the medical device being produced.

The biocompatible articles of the present invention may comprise several biomaterials modified with a non-proteinaceous catalyst for the dismutation of superoxide or a ligand precursor of a non-proteinaceous catalyst for the dismutation of superoxide. This versatility will make these materials especially useful in medical devices that are subject to continual wear and stress, such as joint replacement implants. The polyethylene "socket" polymer portion of the joint which allows a lowered friction contact point in the implant could be injection molded from a copolymer with the non-proteinaceous catalyst, while the metal "ball" portion of the joint which contacts the polyethylene could be surface

covalently conjugated with a non-proteinaceous catalyst. Thus, an entire device with decreased inflammatory response may be manufactured out of the modified biomaterials of the present invention, even though  
5 diverse materials are used in its construction. Another use for the modified biomaterials, mentioned in the stent example above, is coatings.

The chemical reactions described above are generally disclosed in terms of their broadest application to the  
10 preparation of the compounds of this invention. Occasionally, the reactions may not be applicable as described to each compound included within the disclosed scope. The compounds for which this occurs will be readily recognized by those skilled in the art. In all  
15 such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art, e.g., by appropriate protection of interfering groups, by changing to alternative conventional reagents, by routine modification of  
20 reaction conditions, and the like, or other reactions disclosed herein or otherwise conventional, will be applicable to the preparation of the corresponding compounds of this invention. In all preparative methods, all starting materials are known or readily prepared from  
25 known starting materials.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore,  
30 to be construed as merely illustrative, and do not limit of the remainder of the disclosure in any way whatsoever.

#### EXAMPLES

All reagents were used as received without  
35 purification unless otherwise indicated. All NMR spectra were obtained on a Varian VXR-300 or VXR-400 nuclear magnetic resonance spectrometer. Qualitative and

quantitative mass spectroscopy was run on a Finnigan MAT90, a Finnigan 4500 and a VG40-250T using m-nitrobenzyl alcohol (NBA) or m-nitrobenzyl alcohol/LiCl (NBA+Li). Melting points (mp) are uncorrected.

## EXAMPLE 1

## PREPARATION OF COMPOUNDS USED IN TEMPLATE SYNTHESIS

Chemicals, Solvents, and Materials. UV Grade

5 Acetonitrile (015-4) and Water (AH365-4) were obtained from Burdick & Jackson (Muskegon, MI). Isopropanol (27,049-0), R,R-1,2-diaminocyclohexane (34,672-1), 2,6-diacetylpyridine (D880-1), 2,6-pyridinedicarboxaldehyde (25,600-5), and trifluoroacetic acid (T6508) were  
 10 purchased from Aldrich (Milwaukee, WI). 2-(N-morpholino)-ethanesulfonic acid (475893) and its sodium salt (475894) were purchased from Calbiochem (La Jolla, CA).

N-(triphenylmethyl)-(1R, 2R)-diaminocyclohexane:

15 To a solution of (1R,2R)-diaminocyclohexane (250 g, 2.19 mol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (3.5 L) at 0 °C was added, dropwise, a solution of trityl chloride (254 g, 912 mol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (2 L) over 4 h. The resulting mixture was allowed to warm to RT and stirred overnight.  
 20 The reaction mixture was washed with water until the pH of the aqueous washes was below 8 (4 x 2 L) and dried over Na<sub>2</sub>SO<sub>4</sub>. Filtration and concentration of the solvent afforded 322.5g (99% yield) of N-(triphenylmethyl)-(1R, 2R)-diaminocyclohexane as a glass: <sup>1</sup>H NMR (300 MHz,  
 25 DMSO-d<sub>6</sub>) δ 7.50 (d, J = 7.45 Hz, 6 H), 7.26 (app t, J = 7.45 Hz, 6 H), 7.16 (app t, J = 7.25 Hz, 3 H), 2.41 (dt, J = 10.3, 2.62 Hz, 1 H), 1.70 (m, 1 H), 1.54 - 0.60 (complex m, 8 H). <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) δ 147.2 (s), 128.4 (d), 127.3 (d), 69.9 (s), 59.0 (d), 54.4 (d),  
 30 36.6 (t), 32.5 (t), 24.6 (t), 24.3 (t). MS (LRFAB) m/z = 363 [M + Li]<sup>+</sup>.

Glyoxal bisimine of N-(triphenylmethyl)-(1R, 2R)-diaminocyclohexane:

To a solution of N-(triphenylmethyl)-(1R, 2R)-diaminocyclohexane (322.5 g, 905 mmol) in  
 35 methanol (4 L) was added glyoxal (51.9 ml of a 40 % solution in water, 452.3 mmol), dropwise over 30 min. The resulting mixture was stirred for 16 h thereafter. The

precipitated product was isolated by filtration and dried in vacuo to afford 322.1 g (97 % yield) of the bisimine product as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.87 (s, 2 H), 7.51 (d, J = 8.1 Hz, 12 H), 7.16 - 7.05 (m, 18 H), 2.95 (b m, 2 H), 2.42 (b m, 2 H), 1.98 - 0.81 (complex m, 18 H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) 161.67 (d), 147.24 (s), 147.22 (s), 128.90 (d), 128.81 (d), 127.73 (d), 127.61 (d), 126.14 (d), 73.66 (s), 70.86 (d), 70.84 (d), 56.74 (d), 32.45 (t), 31.77 (t), 24.02 (t), 23.62 (t). MS (LRES) m/z 757 [M + Na]<sup>+</sup>.

N,N'-Bis{(1R,2R)-[2-(Triphenylmethylamino)]cyclohexyl}-1,2-diaminoethane:  
The glyoxal bisimine of N-(triphenylmethyl)-(1R,2R)-diaminocyclohexane (586 g, 798 mmol) was dissolved in THF (6 L) and treated with LiBH<sub>4</sub> (86.9 g, 4.00 mol) at RT. The mixture was stirred for 12 h at RT and treated with a second 86.9 g (4.00 mol) portion of LiBH<sub>4</sub>. The reaction was then warmed to 40 °C for 4 h thereafter. The reaction was carefully quenched with water (1 L) and the THF was removed under reduced pressure. The residual slurry was partitioned between CH<sub>2</sub>Cl<sub>2</sub> (3 L) and water (1 additional L). The layers were separated and the aqueous layer was extracted again with CH<sub>2</sub>Cl<sub>2</sub> (1 L). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried (MgSO<sub>4</sub>), filtered and concentrated to afford 590 g (~100 % crude yield) of N,N'-bis{(1R,2R)-[2-(triphenylmethylamino)]cyclohexyl}-1,2-diaminoethane as a white foam: MS (LRES) m/z 739 [M + H]<sup>+</sup>.

N,N'-Bis{(1R,2R)-[2-(amino)]cyclohexyl}-1,2-diaminoethane tetrahydrochloride: To a solution of N,N'-bis{(1R,2R)-[2-(triphenylmethylamino)]cyclohexyl}-1,2-diaminoethane (590 g, 798 mmol) in acetone (3 L) was added concentrated HCl (1.5 L). The reaction was stirred for 2 h and concentrated. The residue was partitioned between water (2 L) and CH<sub>2</sub>Cl<sub>2</sub> (1 L). The layers were separated and the aqueous layer was concentrated and dried in vacuo to afford 257 g (80 % yield) of the

tetrahydrochloride salt as a granular off-white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 3.82-3.57 (complex m, 8 H), 2.42 (d, J = 9.9 Hz, 2 H), 2.29 (d, J = 9.3 Hz, 2 H), 2.02-1.86 (complex m, 4 H), 1.79-1.60 (complex m, 4 H), 1.58-1.42 (complex m, 4 H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) 59.1 (d), 51.3 (d), 40.8 (t), 29.2 (t), 26.0 (t), 22.3 (t), 22.2 (t). MS (LRFAB) m/z 255 [M + H]<sup>+</sup>. The tetrahydrochloride salt can be recrystallized or precipitated from a viscous aqueous solution by the addition of ethanol. This treatment removed all color.

## EXAMPLE 2

### TEMPLATE SYNTHESIS OF COMPOUND 38

[Manganese (II) dichloro{(4R,9R,14R,19R)-3,10,13,20,26-pentaazatetracyclo[20.3.1.04,9.014,19]hexacos-1(26),22(23),24-triene}}].  
In a 5-L flask N,N'-Bis {(1R,2R)-[2-(amino)]cyclohexyl}-1,2-diaminoethane tetrahydrochloride, (93.5 g, 234 mmol), was suspended in ethanol (3 L), treated with solid KOH (59.6 g of 88% material, 934 mmol), and the resultant mixture stirred at RT for 1 h. MnCl<sub>2</sub> (anhydrous, 29.4 g, 233.5 mmol) was then added in one portion and the reaction was stirred at RT for 15 min. To this suspension was added 2,6-pyridinedicarboxaldehyde (31.6 g, 233.5 mmol) and the resulting mixture was refluxed overnight. After 16h, the template reaction was complete: MS (LRFAB) m/z 443 [M - Cl]<sup>+</sup>. See accompanying HPLC analyses. This material was taken on to the next step "as is". The reaction mixture containing the template product in ethanol was cooled to RT and treated (cautiously under Argon flow) with 10 % Pd(C) (~100 g in portions over the next 3-4 days) and ammonium formate (~200 g also in portions over the next 3-4 days). The reaction was refluxed for 4 days. HPLC and MS analysis at this time showed complete reduction. The catalyst was filtered through celite and the filtrate was concentrated

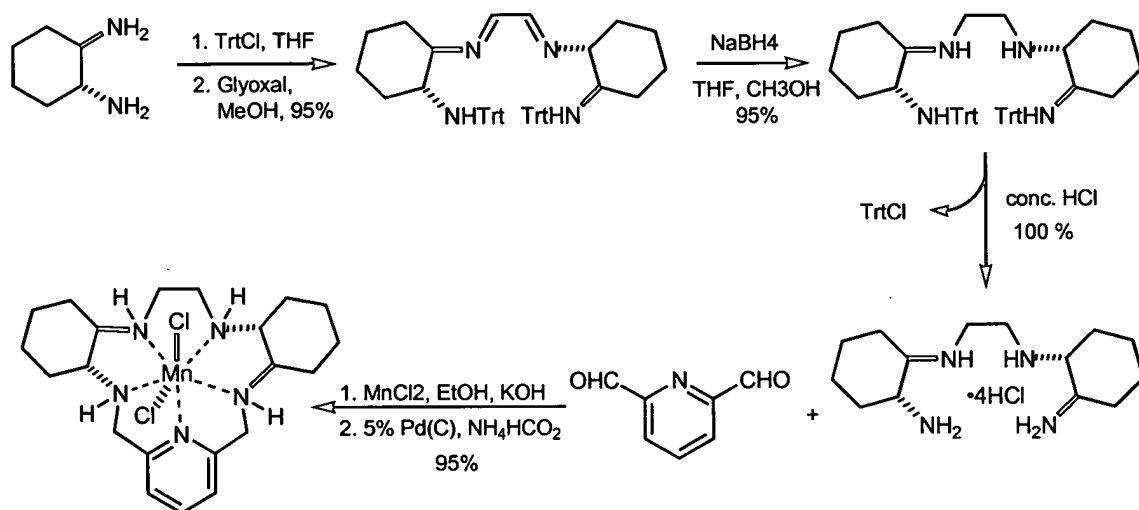


to afford ca. 110 g of crude material.

Recrystallization from water afforded 50.0 g of the product in crop one as a pale yellow finely divided solid. Upon sitting a second crop (12.5 g) was isolated.

- 5 MS (LRFAB)  $m/z$  447  $[M - Cl]^+$ . After drying the combined crops overnight in vacuo at 70°C, a yield of 60.1 g (54%) was obtained. Analysis calc'd for  $C_{21}H_{35}Cl_2N_5Mn$ : C, 52.18; H, 7.30; N, 14.49; Cl, 14.67. Found: C, 51.89; H, 7.35; N, 14.26; Cl, 14.55.

10



The synthesis is diagramed below:

### EXAMPLE 3

15

#### TEMPLATE SYNTHESIS OF COMPOUND 40

- 20 [Manganese(II) dichloro(4R,9R,11R,14R,19R) - 3,10,13,20,26-pentaaza - (2R,21R) - dimethyltetracyclo[20.3.1.0<sup>4,9</sup>.0<sup>14,19</sup>]hexacose- 1(25),22(26),23-triene, To a stirred solution of  $N,N'$ -Bis{(1R,2R) - [2-(amino)]cyclohexyl}-1,2-diaminoethane tetrahydrochloride (4.00 g, 10.0 mmol) in absolute ethanol (100 mL) was added KOH (2.55 g of ~88% material, 40.0 mmol) and the mixture was stirred at RT for 30 min.

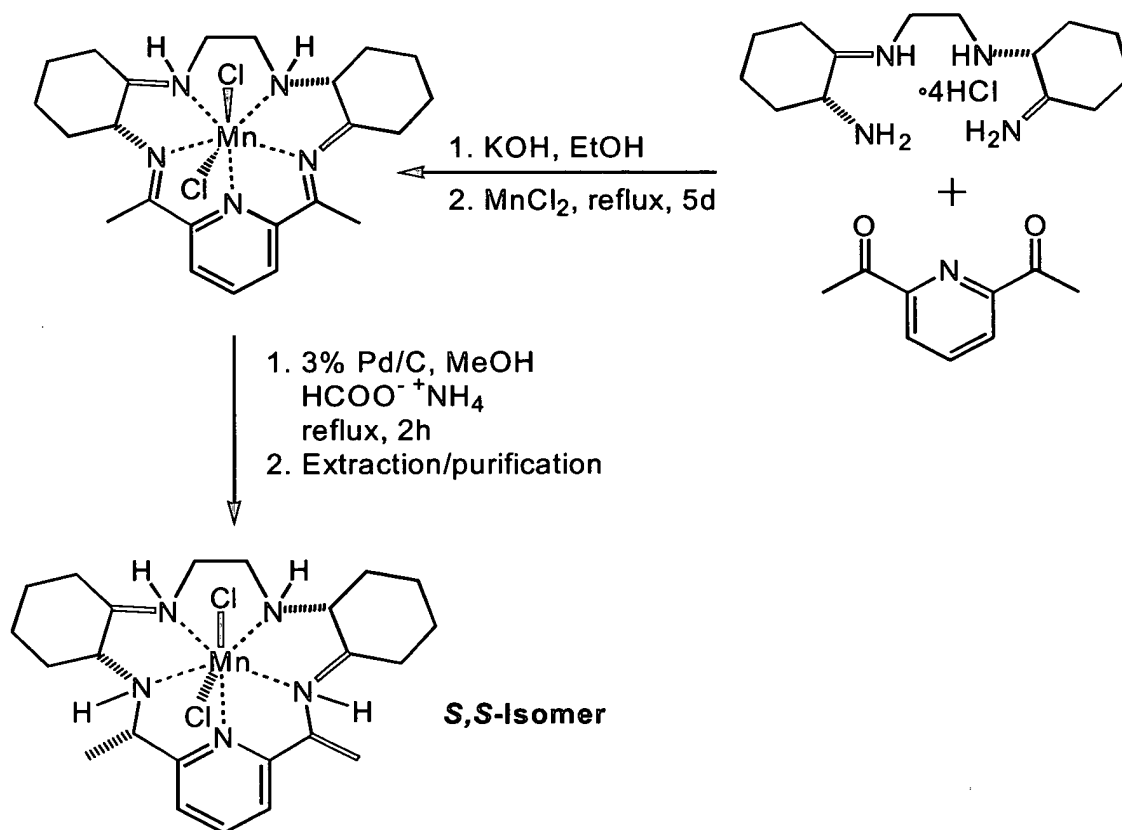
under an Ar atmosphere.  $\text{MnCl}_2$  (anhydrous, 1.26 g, 10.0 mmol) was then added and the suspension stirred for an additional 30 min. or until  $\text{MnCl}_2$  dissolved. At this point, 2,6-diacetylpyridine (1.63 g, 10.0 mmol) was  
5 added to the green mixture and after 30 minutes heating commenced. After refluxing for 5 d, the mixture was dark red-brown. Mass spectrometry and HPLC analyses showed that the reaction had gone to 95% completion to give the bisimine Mn(II) complex (~94% purity by HPLC): ESI-MS:  
10 m/z (relative intensity) 471/473 (100/32)  $[\text{M} - \text{Cl}]^+$ ; only traces of diacetylpyridine (~5% by HPLC) and unreacted tetraamine complex (MS) were detected. The suspension was allowed to cool to RT, and was stirred overnight. The  
15 next day, the suspension was filtered (largely KCl) and dried in vacuo at 70 °C overnight. This material may be further purified by extractive work-up as follows: 69 g of the crude bisimine were dissolved in 1.2 L of distilled water. The yellow-orange solution was extracted with  $\text{CH}_2\text{Cl}_2$  (4 x 500 mL) and then 210 g of NaCl were added  
20 (final solution is ~15% w/v in NaCl). The resulting suspension was extracted with  $\text{CH}_2\text{Cl}_2$  (4 x 500 mL). The combined extracts were pooled, dried over  $\text{MgSO}_4$ , filtered, and the solvent removed under reduced pressure. Upon drying in vacuo at 70 °C overnight, the product was  
25 isolated as an amorphous orange solid (ca. 50g, 78% recovery) with a purity of ca. 98% by HPLC.

Transfer hydrogenation with ammonium formate.

The purified bisimine (1.0 g, 1.97 mmol) was  
30 dissolved in 100 mL of anhydrous MeOH and the flask flushed with nitrogen while 3% Pd/C (0.5 g, 50% by weight) was added. The suspension was heated and 10 mL of a MeOH solution containing ammonium formate (1g, 16 mmol) were added. After 30 and 60 min. of reflux, a second and  
35 third portion of formate were added (16 mmol each). The suspension was allowed to cool to RT after 2h of reflux (at this point the supernatant was nearly colorless),

filtered through celite and the solvent removed under reduced pressure. The resulting yellow-green semisolid was stirred with 50 mL of  $\text{CH}_2\text{Cl}_2$  for 5-10 min., filtered, and the solvent removed once more. The remaining yellow-green foam consisted of ~95% *S,S*- and *S,R*-isomers in a 3.8:1 ratio as determined by HPLC.

10 The synthesis is diagramed below:



Purification protocol.

15 Extraction of Compound 40 (*S,S*-isomer) from the crude mixture obtained from transfer hydrogenation.

Crude product isolated after transfer hydrogenation (9.3 g) was dissolved in water (370 ml) and extracted with DCM (4 x 185 ml). All organic extracts and aqueous phase were analyzed by HPLC to follow the progress of  
5 extraction. Analysis was performed either in a complex form or after release of the free ligand. Recovery of R,S- and S,S-isomer from DCM (1+4, extracts from water): (2.42g +1.18g+1.24g = 4.84 g). After 4 extraction with DCM no R,S-isomer was detected by HPLC in aqueous  
10 phase. Then solid NaCl was added (10.82 g) to make up 0.5 M solution and S,S-complex (Compound 40) was extracted 4 times with DCM (370 ml each). Most of the S,S-isomer was extracted into the 1<sup>st</sup> DCM extract (purity by HPLC>94%). Impurities (others than R,S-isomer) were  
15 extracted at 4-6 % level). After evaporaion of the first two DCM extracts and drying under high vacuum , 3.04g of S,S-isomer Compound 40 was obtained with purity 94%. The product was further purified by HPLC using YMC C18 column or by flash chromatography over C18  
20 silica gel column.

#### Purification by preparative HPLC

Compound 40 (200 mg) obtained from extraction  
25 (purity 91 %) was dissolved in water (1.0 ml) and applied onto YMC CombiPrep column (20mm x 50mm, ODS AQ 5um 120A). The product was eluted using gradient - B 10 to 50 % in 10 min, where A: 0.5M NaCl and B: Acetonitrile-Water (4:1), flow rate 25 ml/min, detection  
30 at  $\lambda=265$  nm. Fractions with purity > 99% (8 to 20, each 5 ml) were combined and the solvents were evaporated to dryness. The residue was partitioned between 6 ml water and 10 ml of DCM. Seperated layers, extracted aqueous layer with 3 x 10ml DCM. Combined DCM layers, dried over  
35 Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated solvents to off-white foam, Obtained 97mg, 48%. ESMS m/z 475 [M-Cl]<sup>+</sup> Calcd for C<sub>23</sub>H<sub>39</sub>Cl<sub>2</sub>N<sub>5</sub>Mn.

Purification of Compound 40 by flash chromatography over C18 silica gel

- 5           40 g of Bakerbond Octadecyl C<sub>18</sub> packing was packed  
into a 25 mm X 130 mm column. Column was equilibrated  
with CH<sub>3</sub>CN (300 ml), 1:1=H<sub>2</sub>O:CH<sub>3</sub>CN (200 ml), 15% CH<sub>3</sub>CN in  
H<sub>2</sub>O(200 ml) and 15 % CH<sub>3</sub>CN in 0.5 M NaCl (200 ml).  
Compound 40 (1 g) obtained from extraction (purity 94 %  
10 by HPLC) was dissolved in 3 ml of H<sub>2</sub>O and applied onto  
the column. The product was eluted with 15% CH<sub>3</sub>CN in 0.5  
M NaCl. Fractions were analyzed by HPLC. HPLC conditions  
were as follows: YMC C<sub>18</sub> column, 3 ml/min,  $\lambda$ =265 nm, B=  
10-50% in 9 min, where A= 0.5 M NaCl in H<sub>2</sub>O and B= CH<sub>3</sub>CN :  
15 H<sub>2</sub>O=4:1. The S,S-isomer eluted in fractions 51-170.  
Fractions with purity > 95% (80-170) were combined and  
the solution was concentrated to 80 ml and extracted 2  
x with DCM. (40 ml each). Obtained 0.64 g (yield 64%)  
of the S,S -isomer (Compound 40), 100% pure by HPLC .  
20 ESMS m/z 475 [M-Cl]<sup>+</sup> Calcd for C<sub>23</sub>H<sub>39</sub>Cl<sub>2</sub>N<sub>5</sub>Mn.

#### EXAMPLE 4

##### TEMPLATE SYNTHESIS OF COMPOUND 42

- 25 Synthesis of 4-chloro-2,6-pyridinedicarboxaldehyde.

4-Chloro-2,6-dicarbomethoxypyridine: Anhydrous chelidamic acid (230 g, 1.14 mol) was partially dissolved in CHCl<sub>3</sub> (2 L) while stirring under N<sub>2</sub>. Then, over a period of 3h, PCl<sub>5</sub> (1,000 g, 4.8 mol) was added as a solid to the cream-colored suspension. Considerable gas evolution occurred with each solid addition. After 17h, the white mixture was heated to reflux and a light yellow solution resulted within an hour. Seven hours later, heating was discontinued. The light suspension was treated with MeOH (1.25 L), added dropwise over 6.5h. Then, after gas evolution had ceased, the solution was concentrated under reduced pressure and the off-white slurry that formed added to deionized water and vacuum-filtered. The residue was washed with more water (~5 L) until the pH of the filtrate was neutral. The residue was dried overnight in vacuo at 50-60 °C to afford 4-chloro-2,6-dicarbomethoxypyridine as white needles (175g, 66%); m.p. 132-134 °C. <sup>1</sup>H-NMR is consistent with the structure.

4-Chloro-2,6-pyridinedimethanol: The methyl ester prepared as above (675 g, 2.94 mmol) was partially dissolved in MeOH (16 L) and stirred under N<sub>2</sub> with cooling in an ice bath. NaBH<sub>4</sub> (500 g, 13.2 mol) was added as a solid in portions over the next 20h. Over the course of 48h, the reaction went from orange to red to yellow-green. Then, the temperature was allowed to reach RT overnight. After this period, the mixture was refluxed for 16h, then cooled over 6h to afford a clear yellow-green solution. Acetone (3.1 L) was added over 1.5h, then the yellow solution was refluxed for 2h. Concentration under reduced pressure yielded an amorphous light yellow gum. The gum was taken up in saturated Na<sub>2</sub>CO<sub>3</sub> and heated to ~80 °C for 1h. Upon cooling overnight, the viscous yellow supernatant was separated from the white precipitate by vacuum filtration. The solid was washed with CHCl<sub>3</sub> (350 mL), then taken in THF (4.5 L) and refluxed for 30 min., then filtered. The filtrate was concentrated under removed pressure, the solid residue

washed with  $\text{CHCl}_3$ , then dried in vacuo overnight to afford the diol product (375 g, 68%) as a white solid.  $^1\text{H-NMR}$  is consistent with the structure.

4-Chloro-2,6-pyridinedicarboxaldehyde: A solution of oxalyl chloride (110 mL, 1.27 mol) in CH<sub>2</sub>Cl<sub>2</sub> (575 mL) was cooled to -60 °C and stirred under N<sub>2</sub>. To this solution was added a solution of dimethylsulfoxide (238 mL, 3.35 mol) in CH<sub>2</sub>Cl<sub>2</sub> (575 mL) via cannula. Addition proceeded with vigorous gas evolution and a mild exothermic reaction over 1.5h. After stirring for 10 min. a solution of the diol (100 g, 0.58 mol) in DMSO (288 mL) was added via cannula over a period of 30 min. The previously yellow solution turned into a suspension. After 2h at -60 °C, Et<sub>3</sub>N (1.5 L) was added dropwise over 1h. After addition was complete and 30 min. had passed, the mixture was poured over water (2 L), shaken and allowed to settle. The organic layer was separated and the aqueous layer extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 x 300 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> layers were dried over MgSO<sub>4</sub> and concentrated under reduced pressure to afford a combination of gray-yellow solid and a reddish liquid. The dark yellow solid was collected by filtration using Et<sub>2</sub>O to rinse out. This material was dissolved in 1L of CH<sub>2</sub>Cl<sub>2</sub> and passed through a bed of SiO<sub>2</sub> (~800 cm<sup>3</sup>) eluting with more CH<sub>2</sub>Cl<sub>2</sub>. A total of 65 g (67%) of the dialdehyde product were collected in this fashion. <sup>1</sup>H-NMR is consistent with the structure.

25

Preparation of 4-chlorobisimine by template cyclization.

Bis-R,R-Cyclohexane tetraamine·4HCl (2.57g, 6.42 mmol) was suspended in absolute EtOH (64 mL) and stirred under Ar. Pellets of KOH (1.65g of 87.4% material, 25.68 mmol) were added and the suspension stirred for 30 min. until the pellets dissolved. After this period, MnCl<sub>2</sub> (anhydrous, 0.806g, 6.42 mmol) was added and allowed to stir for 1-2h until the suspension turned greenish and all the MnCl<sub>2</sub> dissolved. 4-Chloro-2,6-pyridinedicarboxaldehyde (1.09g, 6.42 mmol) was added as a solid and the mixture stirred at room temperature for



30 min., then heated to reflux. The suspension gradually turns red-orange and after 48 hours it was cooled to room temperature. The mixture was filtered through a 10m pore-size funnel and the solvent removed under reduced  
5 pressure to yield the desired product (3.47g, 105%, contains some inorganic salts) as a red-orange solid.

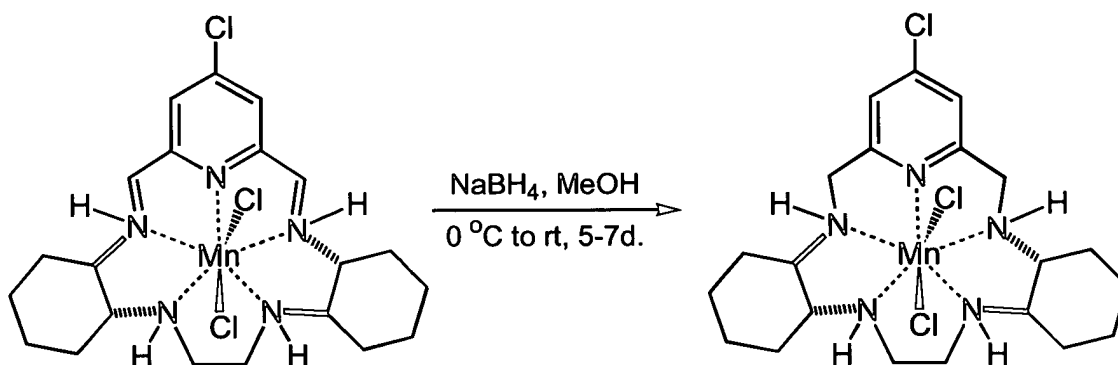
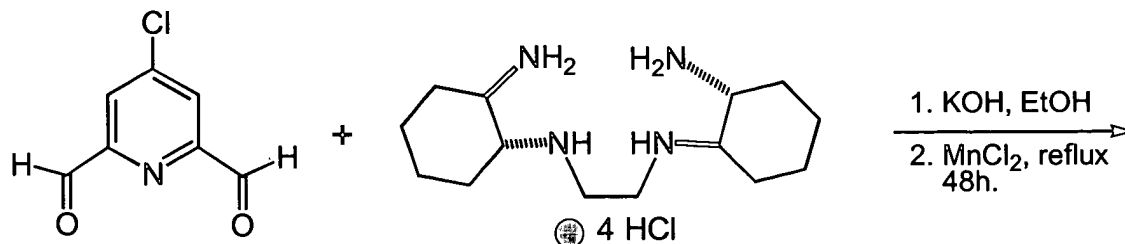
NaBH<sub>4</sub> Reduction.

The bis-imine complex (1.89g, 3.68 mmol) was dissolved in anhydrous MeOH (50 mL) and stirred under Ar in an ice-water bath. Solid NaBH<sub>4</sub> (0.278g, 7.36 mmol) was added in one portion resulting in gas evolution. After 30 min., an additional portion of NaBH<sub>4</sub> (7.36 mmol) was added and the mixture allowed to warm to RT, and stirred overnight. A third portion of NaBH<sub>4</sub> (7.36 mmol) was added at 0EC, then the mixture allowed to warm and stirred overnight. After this period, MS still showed starting material remaining. A fourth, fifth, and sixth portion of NaBH<sub>4</sub> (7.36 mmol each) were added with 2 hours passing in between addition.

After 24h at RT, the lightly colored solution was carefully added onto 100 mL of saturated NaCl solution, and MeOH removed under reduced pressure. CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added and the aqueous layer extracted(2X). The organic layers were combined, dried over MgSO<sub>4</sub>, filtered and the solvent removed to afford, upon drying in vacuo, 2.1 g of crude material (60% product by HPLC). This material was purified by SiO<sub>2</sub> flash chromatography using 1 à 3% MeOH:CH<sub>2</sub>Cl<sub>2</sub> as eluent. Selected fractions yielded 0.77 g (40%) of HPLC-homogeneous material. ESI-MS: m/z (relative intensity) 481/479 (100/32) [M-Cl]<sup>+</sup>; and 223/221 (100/32) [M-2Cl]<sub>2</sub><sup>+</sup>.

30

The synthesis is diagramed below:



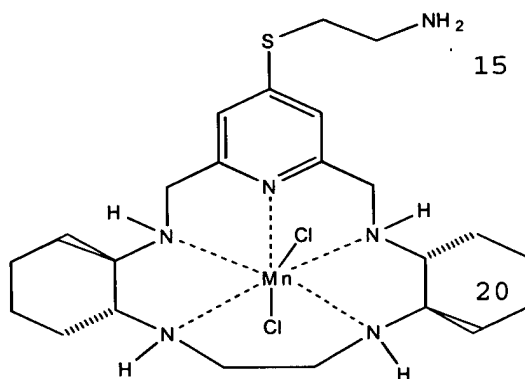
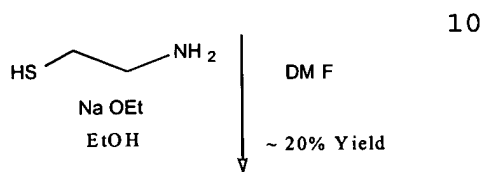
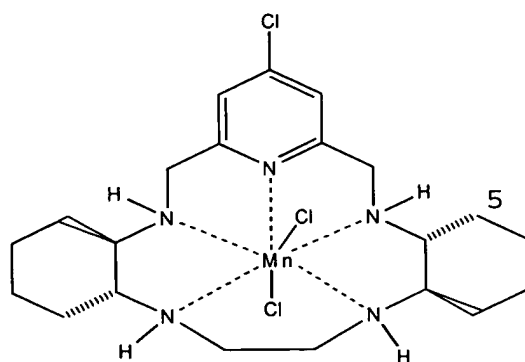
## EXAMPLE 5

## SYNTHESIS OF COMPOUND 43 FROM COMPOUND 42

5

To a solution of 1.2 % (w/v) 2-mercaptoethylamine (1 eq) in ethanol at 0 °C was added sodium ethoxide (1.1 eq) to generate the thiolate. After stirring for 1.75 h, the thiolate solution was added dropwise to a solution of 1.3 % (w/v) SC 74897 (1 eq) in DMF at 0 °C. The reaction mixture was allowed to stir overnight. The solvent was removed *in vacuo*, the product mixture was extracted with methylene chloride, and concentrated down *in vacuo*. Flash column chromatography using methylene chloride:methanol (9:1) as the eluent was used for purification, which was monitored via HPLC.

The synthesis is illustrated below:



## EXAMPLE 6

25

## CATALYTIC HYDROGENATION OF THE BISIMINE

Transfer hydrogenation with ammonium formate.

The purified bisimine (1.0 g, 1.97 mmol) was dissolved in 100 mL of anhydrous MeOH and the flask  
 30 flushed with nitrogen while 3% Pd/C (0.5 g, 50% by weight) was added. The suspension was heated and 10 mL of a MeOH solution containing ammonium formate (1g, 16 mmol) were added. After 30 and 60 min. of reflux, a second and third portion of formate were added (16 mmol each). The  
 35 suspension was allowed to cool to RT after 2h of reflux (at this point the supernatant was nearly colorless), filtered through celite and the solvent removed under

reduced pressure. The resulting yellow-green semisolid was stirred with 50 mL of  $\text{CH}_2\text{Cl}_2$  for 5-10 min., filtered, and the solvent removed once more. The remaining yellow-green foam consisted of ~95% S,S- and S,R-isomers in a  
5 3.8:1 ratio as determined by HPLC.



EXAMPLE 7  
CONJUGATION OF POLYETHYLENE TEREPHTHALATE  
WITH A PACPeD CATALYST

5    A. Denier reduction (alkaline hydrolysis) of Poly(ethylene terephthalate) (PET) film

20 mm X 50 mm X 5 mm PET film (37 % crystallinity) pieces were cleaned by mixing for 30 min in a 1 % (w/w) aqueous  $\text{Na}_2\text{CO}_3$  solution (250 mL) at 75°C. The film pieces  
10 were removed and washed 30 min in water (HPLC grade, 250 mL) at 75°C. The pieces were next hydrolyzed for 30 min in a 0.5 % (w/w) aqueous NaOH solution (250 mL) at 100°C. The film pieces added to a 1.2 % (w/w) aqueous conc. HCl  
15 pieces were thoroughly rinsed in a stream of water (HPLC grade) at room temperature and dried to constant weight in vacuo.

B. Preparation of the acid chloride

20        A magnetic stir bar and anhydrous acetonitrile (50 mL) were added to a dry 100 mL round bottom flask. To the stirring solvent was added one piece of hydrolyzed film, pyridine (0.078 g,  $9.89 \times 10^{-4}$  mol), and thionyl chloride (0.167 g,  $1.4 \times 10^{-3}$  mol). After stirring for 24 h at room  
25 temperature, the film was removed and thoroughly rinsed in fresh acetonitrile. After drying to constant weight in vacuo, elemental analysis showed the presence of chlorine in the film.

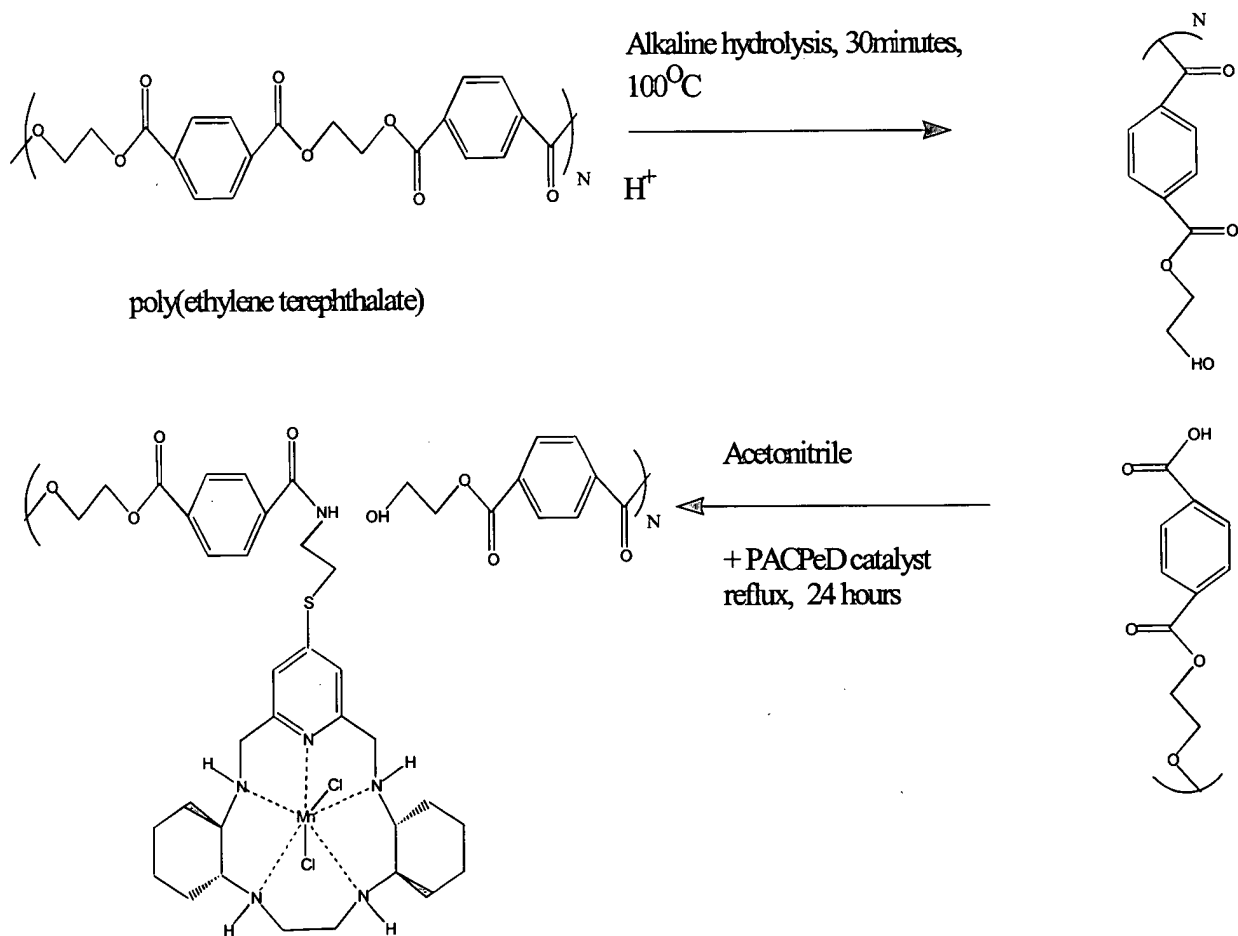
30    C. Reaction with amino functional PACPeD

A magnetic stir bar and anhydrous acetonitrile (50 mL) were added to a dry 100 mL round bottom flask. Amino functional Compound 43 ( $0.138$  g,  $1.86 \times 10^{-4}$  mol) was added. Once in solution, the film step B was added and the reaction

mixture was heated to reflux. After 24 h at reflux, the film was removed and rinsed in fresh acetonitrile before drying to constant weight *in vacuo*. ICAP analysis of the film revealed the presence of manganese.

5

The conjugation scheme is illustrated by the following:





EXAMPLE 8  
CONJUGATION OF ACRYLIC ACID MODIFIED POLYETHYLENE WITH A  
PACPeD CATALYST

5    A. Grafting of acrylic acid to PET films

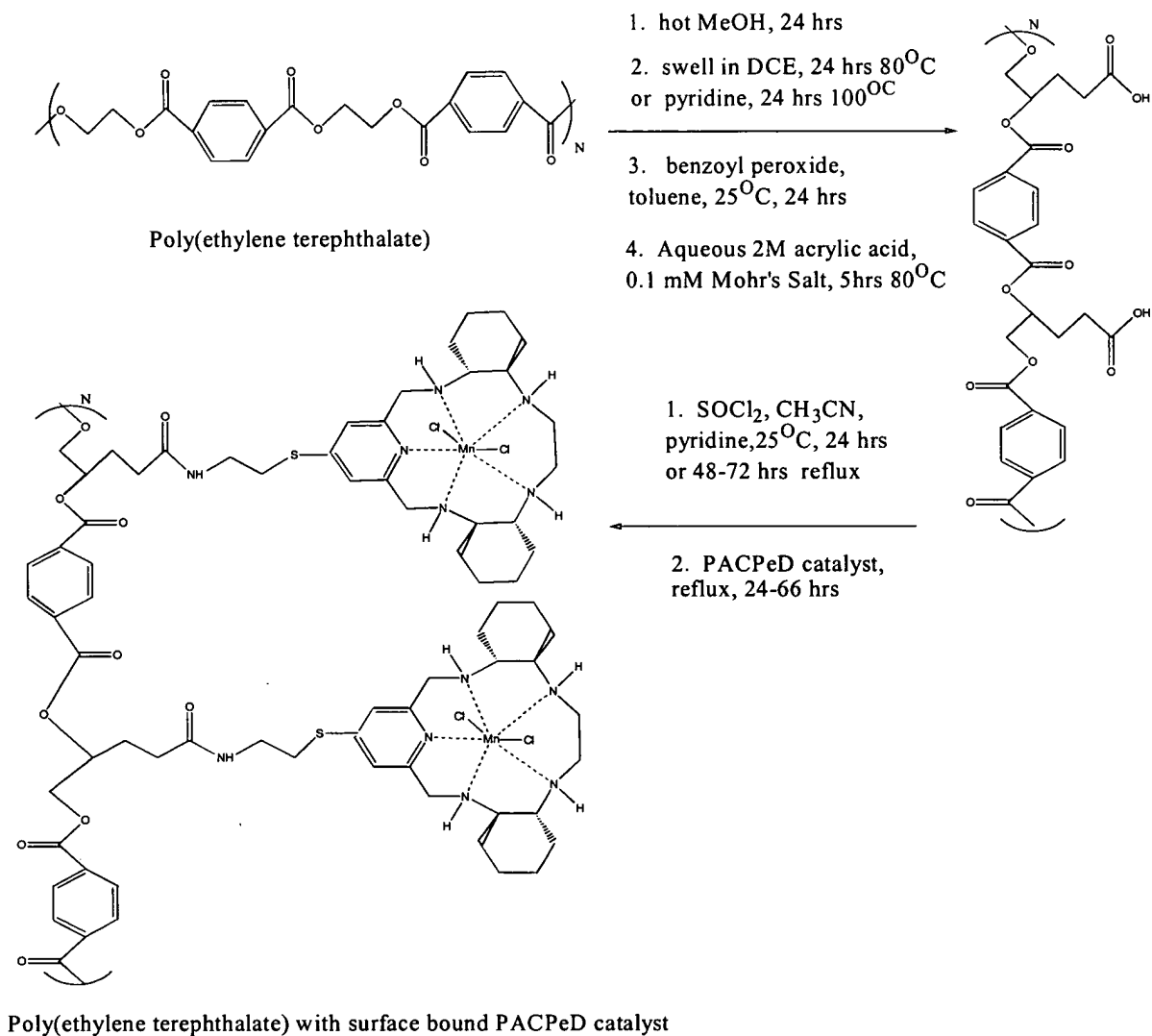
Pieces of 20 mm X 50 mm X 5 mm PET film (37% crystallinity) were used without cleaning. Film pieces were swollen in 80°C 1,2-dichloroethane for 1 h. The films were then dried to constant weight *in vacuo*.

10        Swollen film pieces were added to a 0.08 M benzoyl peroxide in anhydrous toluene solution (125 mL). After mixing for 1 h at room temperature, the film pieces were removed, rinsed in fresh anhydrous toluene, and dried to constant weight *in vacuo*.

15        Next, the films were immersed in a 30 mL vial containing a 2 M acrylic acid (freshly distilled) and 0.1 mM Mohr's salt  $\{(NH_4)_2Fe(SO_4)_2 \cdot 6 H_2O\}$  aqueous solution (25 mL). The vial was purged with nitrogen, sealed, and immersed in an 80°C oil bath. The film pieces were stirred  
20    for 20-24 h at 80°C before removal and rinsing for several minutes in hot running tap water followed by a stream of room temperature water (HPLC grade). After drying overnight *in vacuo*, the acrylic acid grafted films were immersed for 5 h in boiling water (HPLC grade) and dried to  
25    constant weight *in vacuo*.

Preparation of the hydrolyzed PET film and conjugation with the PACPeD catalyst proceeded as described in Example 7.

30    The conjugation scheme is illustrated by the following:



## EXAMPLE 9

5 SURFACE COVALENT CONJUGATION OF COMPOUND 43 WITH  
POLY (ETHERURETHANEUREA)

The poly(etherurethaneurea) (PEUU) ( $M_n = 50,000$ ) used for conjugation was a segmented block copolymer consisting of methylene di(*p*-phenyl isocyanate) (MDI), ethylene  
10 diamine, and poly(tetramethyleneglycol) (PTMG,  $M_n = 2000$ ). The ethylene diamine chain extended MDI makes up the hard

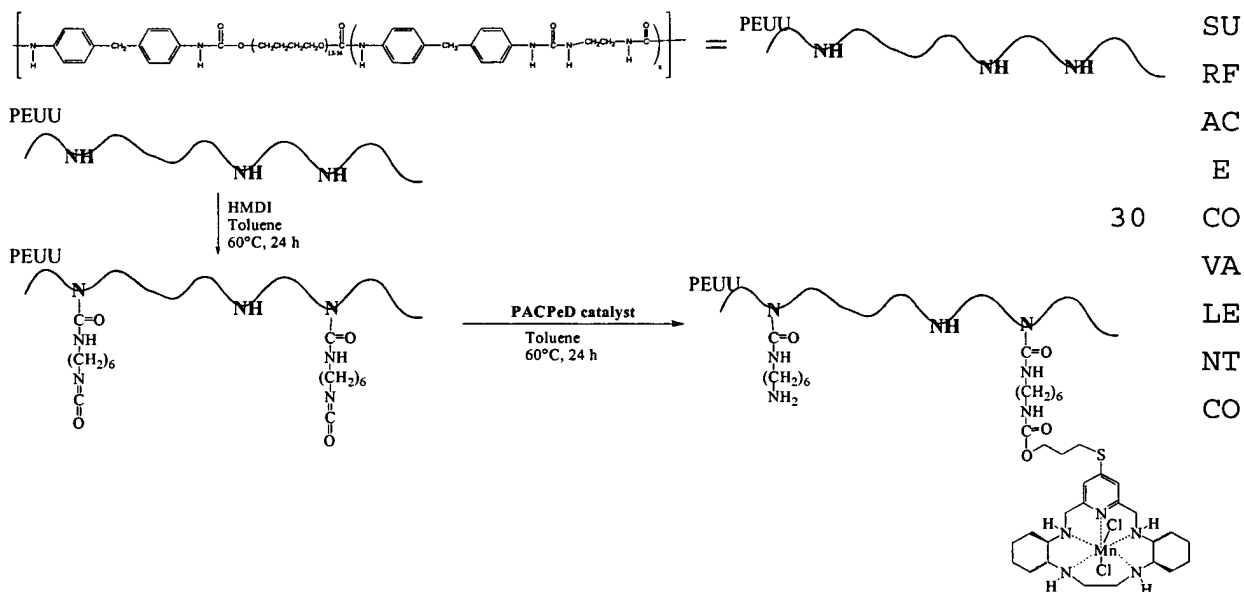
segment and the PTMG makes up the soft segment. PEUU films were solvent cast from a solution of 20 % PEUU in N,N-dimethylacetamide (DMAc) and allowed to dry under nitrogen for ~ 2 days. Films were further dried *in vacuo* before  
5 being cut into ~ 5 mm diameter disks of ~ 0.3 mm thickness.

PEUU disks were functionalized in a solution of 5.4 % (w/v) HMDI in anhydrous toluene with triethyl amine added to serve as the catalyst. The reaction was allowed to stir at 55-60 °C for 24 h, the disks were thoroughly washed with  
10 anhydrous toluene, and dried. Disks were added to a solution of 0.3 % (w/v) Compound 43 in anhydrous toluene and allowed to stir at 55-60 °C for 24 h. The disks were washed with toluene, methanol, and water to remove any unbound SOD mimic prior to implantation. By inductively  
15 coupled argon plasma analysis (ICAP, Galbraith Laboratories, Knoxville, TN) of manganese there was 3.0 % catalyst by weight.

To obtain a lower concentration of Compound 43, a solution of 0.7 % (w/v) HMDI in anhydrous toluene (15 h)  
20 and a solution of 0.1 % (w/v) Compound 43 in anhydrous toluene (24 h) was used. ICAP analysis of manganese indicated 0.6 % Compound 43 by weight.

25

## EXAMPLE 10



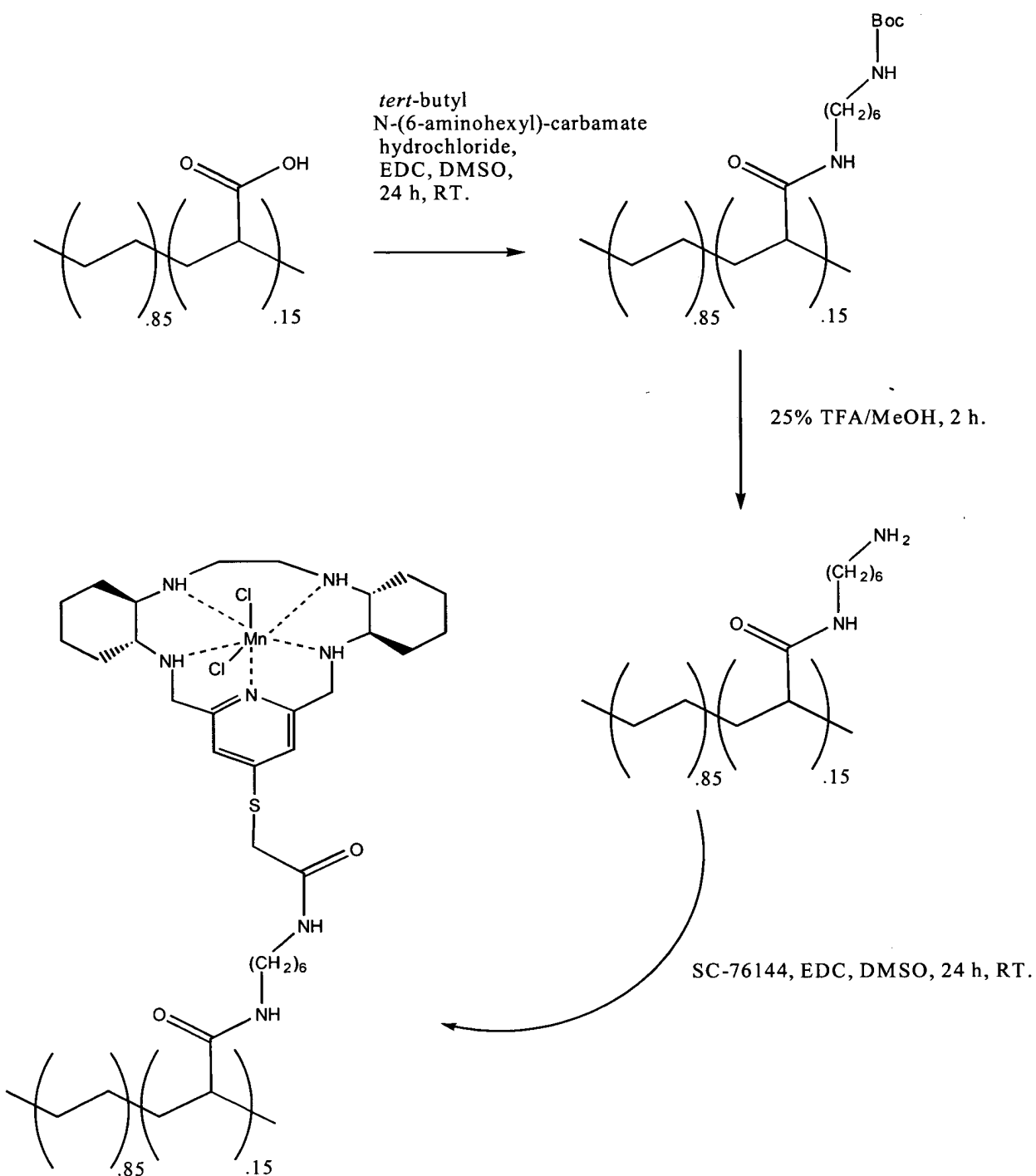
## NJUGATION OF COMPOUND 43 AND POLY(ETHYLENE ACRYLIC ACID)

UHMWPE was melt blended with poly(ethylene-co-acrylic acid) in a ratio of 7:3 in a DACA twin screw at 175 °C.

- 5 Blends were cryoground and melt pressed into films with 5000 psi at 175 °C for 10 minutes. Films were cut into 5 mm diameter disks of ~ 0.5 mm thickness.

- PE disks were chlorinated in a solution of 0.2 % (w/v) thionyl chloride in acetonitrile. Pyridine was added to  
10 scavenge the HCl formed. The mixture was allowed to stir overnight, the disks were filtered, washed thoroughly with acetonitrile, and dried. Chlorinated disks were added to a solution of 0.1 % (w/v) Compound 43 in acetonitrile, heated to reflux for 4 hours, and allowed to react at room  
15 temperature overnight. The disks were filtered and washed with acetonitrile and water. ICAP analysis for manganese indicated 1 % Compound 43 by weight.

- To obtain a lower concentration of Compound 43, the chlorinated disks were added to a solution of 0.02 % (w/v)  
20 Compound 43 in DMSO and heated at 60 °C overnight. The disks were filtered and washed repeatedly with methanol and water. ICAP analysis for manganese indicated 0.06 % Compound 43 by weight.



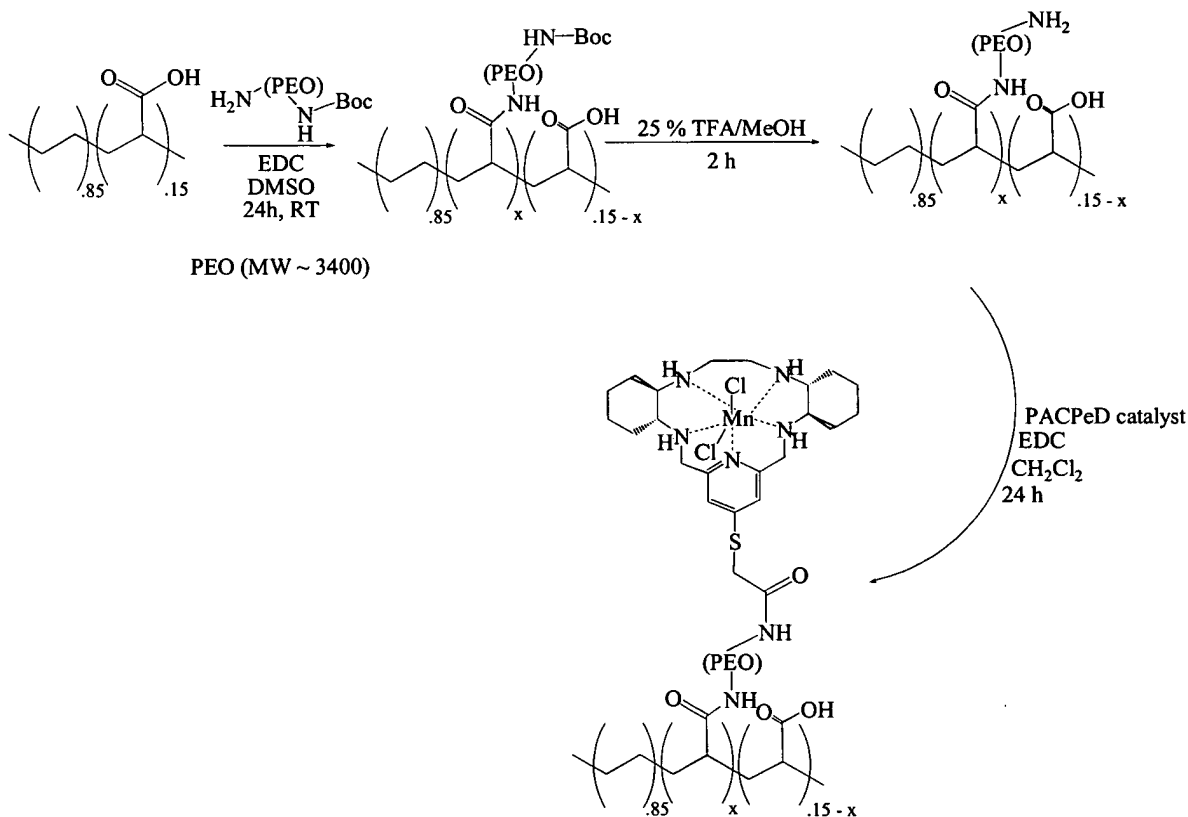
The synthesis is diagramed below:

EXAMPLE 11  
SURFACE COVALENT CONJUGATION OF COMPOUND 52 WITH  
POLY(ETHYLENE-CO-ACRYLIC ACID) VIA A PEO LINKER

5        To a flask under a N<sub>2</sub> purge was added polyethylene-co-  
polyacrylic acid (0.4 g) (15 % acrylic acid by weight),  
DMSO (100 mL), and EDC (0.3192 g). The mixture was allowed  
to stir for 1 h, then polyoxyethylene bisamine (amino-PEO)  
10        (2.65 g) (Sigma, MW = 3400) was added. The mixture was  
allowed to stir overnight. The mixture was precipitated  
with water and dried *in vacuo* yielding 0.37 g of white  
powder. The powder was 1.9 % N by weight as determined by  
elemental analysis.

15        To a flask under a N<sub>2</sub> purge was added EDC (0.0112 g),  
Compound 52 (0.031 g), and CH<sub>2</sub>Cl<sub>2</sub>. The solution was allowed  
to stir for 2 h at room temperature and then the amino  
terminated PEO functionalized polyethylene-co-polyacrylic  
acid (0.2 g) was added and the solution was allowed to stir  
overnight. Methanol (50 mL) was added to the solution, the  
20        precipitate was filtered off, washed with methanol and  
water, and dried *in vacuo* overnight. By ICAP analysis,  
0.26 % of manganese by weight was present.

The synthesis is diagramed below:



5

#### EXAMPLE 12

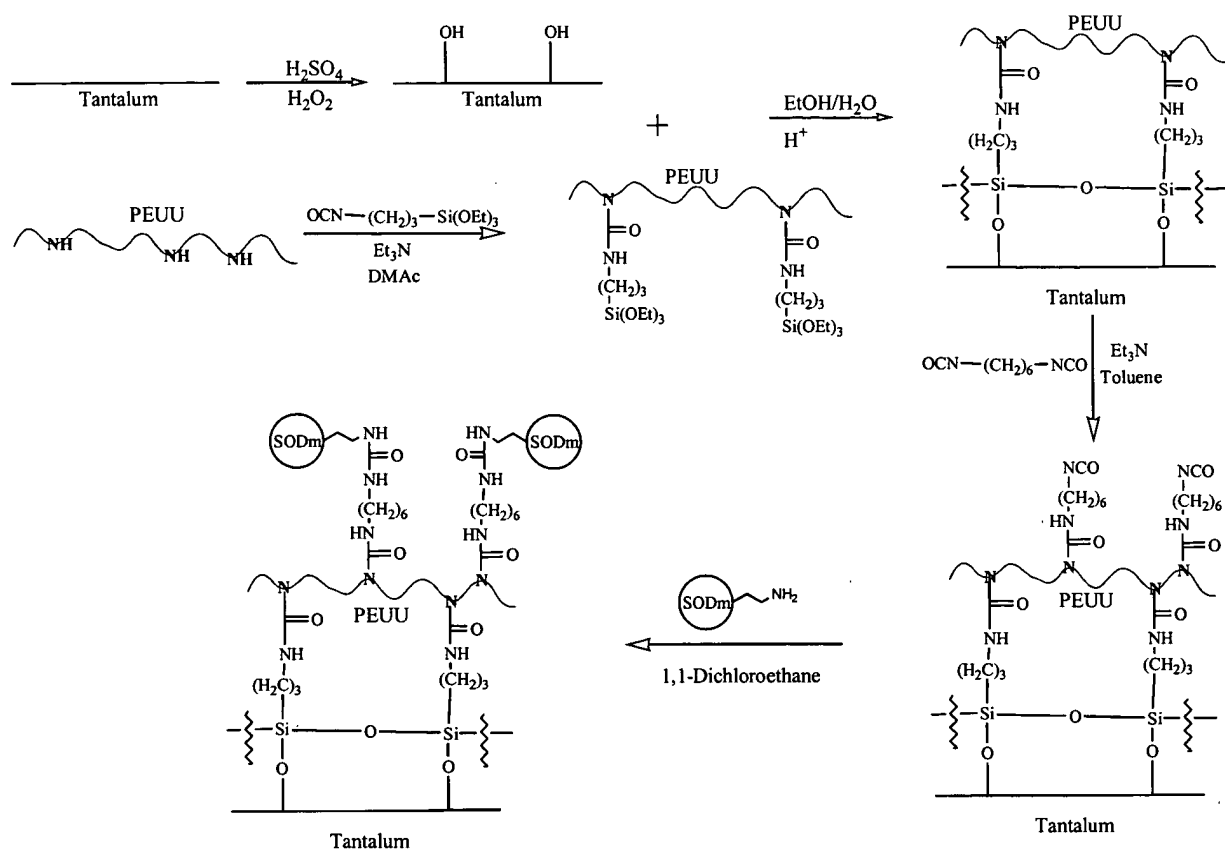
#### SURFACE COVALENT CONJUGATION OF COMPOUND 43 WITH POLY(ETHERURETHANE UREA) COATED TANTALUM

To a 0.5 % (w/v) PEUU solution in DMAc was added 3-  
 10 isocyanatopropyl triethoxysilane (3 % w/v) and triethyl  
 amine. The reaction mixture was heated to 55-60 °C for 18  
 h and then precipitated with ethanol, filtered, and dried.  
 A solution of 1 % (w/v) polymer in DMAc was formed. To the  
 oxidized tantalum disks was added polymer solution and  
 15 water (50:1, v:v). After agitation for 24 h, the disks  
 were cured at 110 °C for 1 h, rinsed with DMAc, and dried.

Half of the disks were set aside for use as controls during implantation. To the PEUU coated disks was added a solution of 5 % (w/v) HMDI in anhydrous toluene and allowed to react at 55-60 °C for 24 h. After washing with  
5 anhydrous toluene and drying, a solution of 1 % (w/v) Compound 43 in 1,1-dichloroethane was added and allowed to react for 24 h at 55-60 °C. The disks were then washed with 1,1-dichloroethane, methanol, and water. After  
10 drying, ESCA was obtained and indicated a 1.2 % atomic fraction of manganese on the surface.

The synthesis is diagramed below:





## EXAMPLE 13

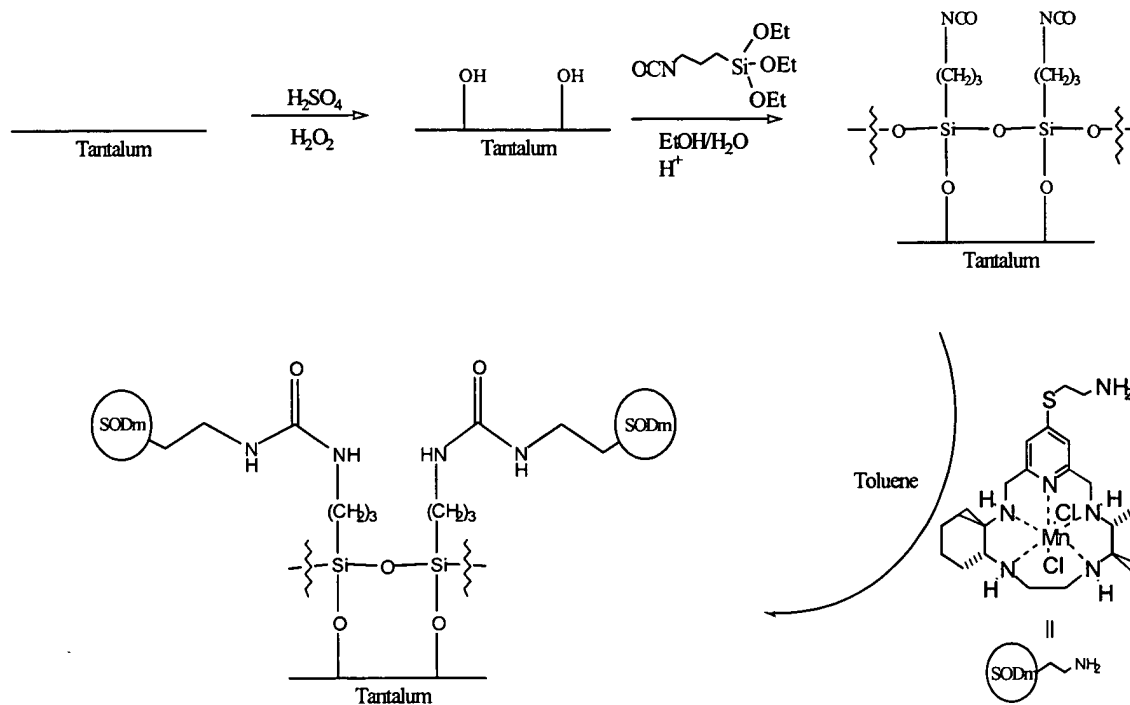
## SURFACE COVALENT CONJUGATION OF COMPOUND 43 WITH TANTALUM

- 5 Disks with a diameter of 6 mm were punched out from 0.25 mm thick tantalum sheets and the edges smoothed.
- Tantalum disks were initially oxidized using a  $\text{H}_2\text{SO}_4$ :30 %  $\text{H}_2\text{O}_2$  (1:1, v:v) solution. 3-isocyanatopropyl triethoxysilane (2 % w/v) was added to an ethanol-water
- 10 solution (0.8 % water by weight) of pH = 5 (adjusted with acetic acid) and agitated for 5 min. To the oxidized tantalum disks was added the silane and after agitation for 10 min, the disks were quickly rinsed with ethanol, and cured at 110 °C for 1 h. Half of the disks were set aside
- 15 for use as controls during implantation. To the polysiloxane layered disks was added a solution of 0.5 %

(w/v). Compound 43 in DMAc and allowed to react at 60-65 °C for 24 h. After washing with DMAc and drying, one disk was studied by electron scanning for chemical analysis (ESCA), which indicated a 0.5 % atomic fraction of manganese on the surface.

The synthesis is diagramed below:

10



#### EXAMPLE 14

#### SURFACE COVALENT CONJUGATION OF COMPOUND 43 WITH COLLAGEN

15 To a flask 0.5g of bovine collagen (insoluble, type I from Achilles tendon) was suspended in a 4% solution of 1,4 butanediol diglycidyl ether in a buffer solution. The solution was stirred overnight. The solution was then

centrifuged for about 10 minutes, and the supernatant was decanted. Any residual, adsorbed diglycidyl ether was removed from the above partially cross-linked collagen by repeated washings with methanol. At this point, the washed  
5 collagen was immersed in a solution of Compound 43 (100mg in 50 ml) of the same buffer used in the reaction set forth above. The contents were stirred at ambient temperature in a round bottomed flask overnight. At the end of this period, the contents were centrifuged and washed as in the  
10 earlier step to remove any unreacted Compound 43. The recovered collagen (0.304g) was dried overnight in a vacuum oven at a temperature of 50° C.

ICAP analysis indicated 0.18% Mn in the collagen corresponding to 1.83% binding of Compound 43.

15

#### EXAMPLE 15

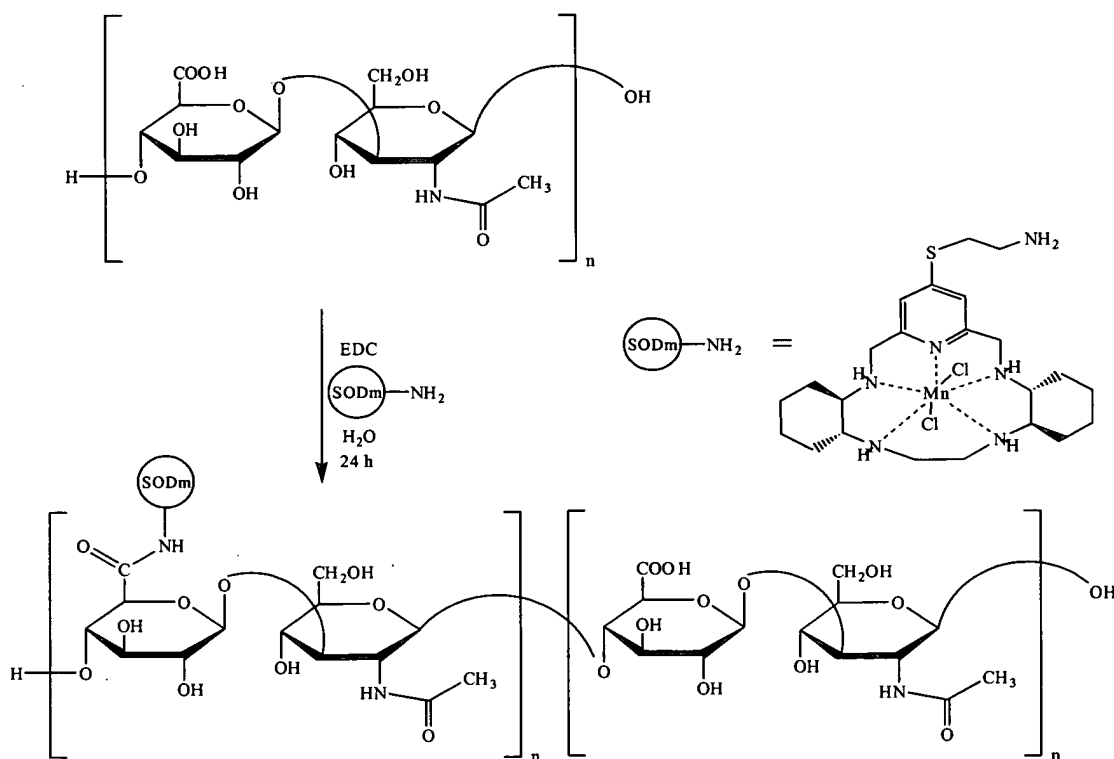
##### SURFACE COVALENT CONJUGATION OF COMPOUND 43 WITH HYALURONIC ACID

20 To a solution of 0.05g of sodium salt of hyaluronic acid (Sigma H53388, Mol.Wt.,  $1.3 \times 10^6$ ) in 16.7ml distilled water was added 0.070g of Compound 43 and the pH of the solution lowered from 9.3 to 6.8 by careful addition of 0.1M HCl. A solution of 1-(3-Dimethylaminopropyl)-3-  
25 ethylcarbodiimide hydrochloride, [EDC.HCl] (0.012g) and 1-Hydroxy-7-azabenzotriazole [HOAT] (0.009g) in dimethylsulfoxide (DMSO)-water (0.5 ml; 1:1, v/v) was added and the pH was adjusted from 5.2 to 6.8 and maintained at 6.8 by incremental additions of 0.1 M sodium hydroxide. The  
30 contents were stirred overnight at ambient temperature. After 20 hr, the pH was readjusted to 6.8 from 6.94 and again stirred overnight for a total of 48 hr. At the end of this period, pH of the solution was again adjusted to 7.0 and dialyzed in Pierce Slide-a-dialyzer cassettes

(Mol.Wt. cutoff:10,000) against distilled water for 65 hr. Dialyzed contents from the cassettes were syringed out (16.7ml) and 0.8g of NaCl was added to obtain a 5% salt solution. The reaction product was precipitated by the addition of ethanol (x3 to 48 ml). The cotton-like white solid was recovered by filtration, dried under vacuum overnight. A total of 0.0523g of the isolated product on ICAP analysis showed a 0.21% Mn corresponding to 2.1% binding of Compound 43 to hyaluronic acid.

10

The synthesis is diagramed below:

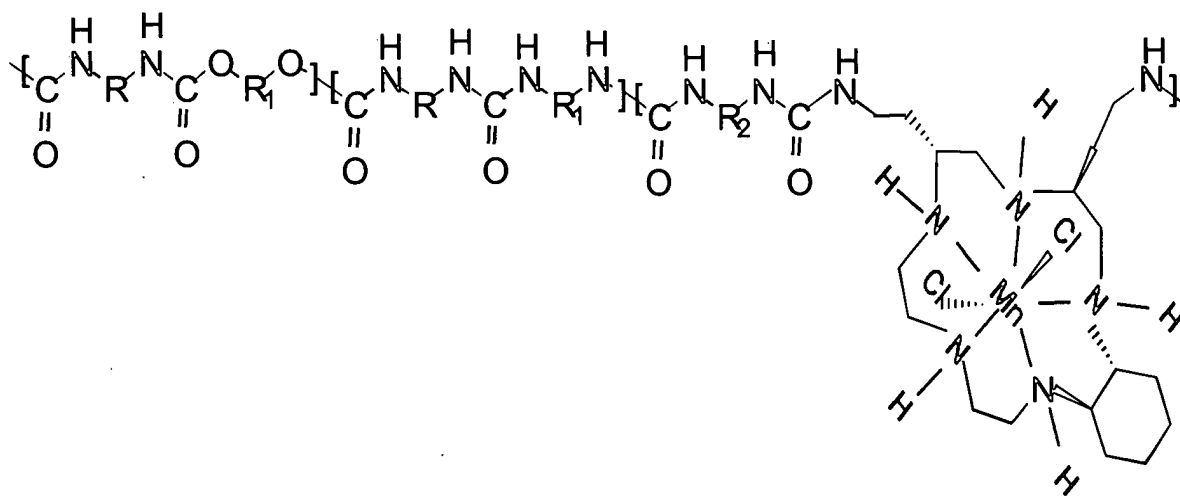


15

EXAMPLE 16  
COPOLYMERIZATION OF COMPOUND 16 WITH POLYUREAURETHANE

A solution of vacuum distilled 4,4'-  
5 methylenebis(phenylene isocyanate) (MDI) is prepared in N,  
N'-dimethylacetamide (DMA). Polytetramethylene oxide  
(PTMO), dehydrated under vacuum at 45-50<sup>E</sup>C for 24 h and  
stannous octoate catalyst are subsequently added to the  
stirred MDI solution at room temperature. The concentration  
10 of the reactants in solution is about 15% w/v and of the  
catalyst is 0.4-0.5% by weight of the reactants. After  
reacting at 60-65<sup>E</sup>C for 1 h, the mixture is cooled to 30<sup>E</sup>C.  
Ethylene diamine (ED) and diamino Compound 16 are then  
added and the temperature gradually brought back to 60-65<sup>E</sup>  
15 C. This is to prevent an excessively rapid reaction of the  
highly reactive aliphatic amine groups with isocyanates.  
The reaction is continued for an additional hour at about  
65<sup>E</sup>C. The entire synthesis is carried out under a  
continuous purge of dry nitrogen. Molar ratios of MDI, ED,  
20 SODm, and PTMO and the molecular weight of PTMO are varied  
to produce polureaurethanes of varying hardness. The  
polymers are precipitated in a suitable non-solvent like  
methanol and dried in a vacuum oven at 70-75<sup>E</sup>C for about a  
week. Films for physical testing and implantation in rats  
25 are prepared by a conventional spin-casting technique  
followed by vacuum drying at 70<sup>E</sup>C for 4 days.

The polymer produced by this method is represented  
diagrammatically below:



## EXAMPLE 17

## COPOLYMERIZATION OF COMPOUND 53 WITH METHACRYLIC

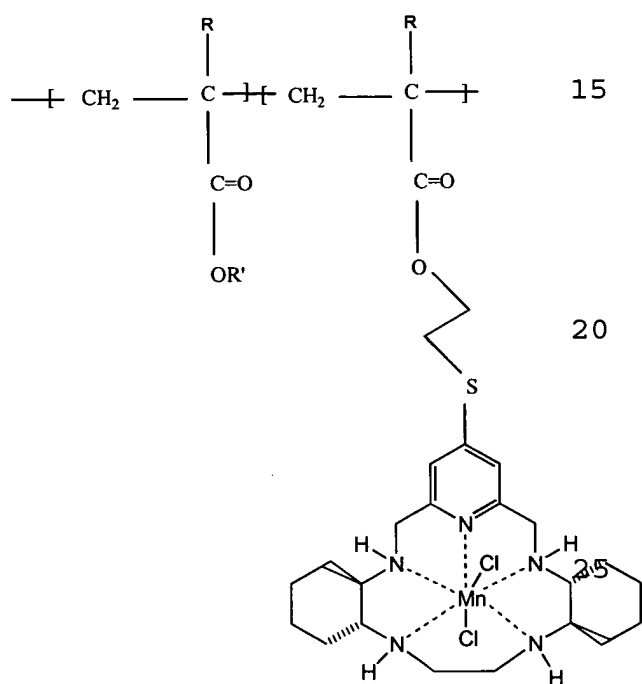
## 5 Synthesis of methacryl functional SODm:

A ~ 10 percent (w/v) solution of hydroxy (or amino) functional PACPeD in 1,2-dichloroethane is placed in a three necked flask equipped with a stirrer, a dropping  
 10 funnel and a reflux condenser. To this solution, a ~10 percent (w/v) solution of methacryloyl chloride in 1,2 dichloroethane is added dropwise at 0°C followed by pyridine. The mixture is stirred at room temperature for about 16 h. The reaction mixture is filtered to remove  
 15 pyridine hydrochloride and the filtrate is concentrated under reduced pressure. The residue is dissolved in methanol and the methacryl functional SODm is recovered by column chromatography.

## 20 Synthesis of (meth)Acrylic copolymers containing SODm:

Mixtures of freshly distilled methyl methacrylate and Compound 53 are dissolved in toluene (~10%w/v) and

transferred to a three necked flask equipped with a stirrer, a nitrogen inlet/outlet and a reflux condenser. Azodiisobutyronitrile (1% on the weight of monomer mixture) is added and the solution is purged free of occluded air by oxygen free nitrogen. The contents are heated to 50°C and maintained at that temperature stirred under a nitrogen sweep for 48 h. The polymer solution is then slowly poured with good stirring into a large excess of methanol to recover the copolymer. The recovered copolymer can be further purified by reprecipitation from a toluene solution in methanol.



This synthesis results in the following polymer:

EXAMPLE 18  
COPOLYMERIZATION OF HEXAMETHYLENE DIAMINE  
WITH COMPOUND 16

5 Synthesis of Poly(hexamethylene -co-SODm sebacamide):

A mixture of hexamethylene diamine (HMD) and diamino  
Compound 16 is dissolved in absolute ethanol and added to a  
solution of sebacic acid in absolute ethanol. The mixing is  
accompanied by spontaneous warming. Crystallization soon  
10 occurs. After standing overnight, the salt is filtered ,  
washed with cold absolute ethanol and air dried to constant  
weight. About 2% excess of HMD is used to promote a salt  
rich in diamine. HMD being the more volatile component, is  
lost during salt drying or during polycondensation.

15

The dried salt is heated in a suitable reactor with  
good stirring first to 2150EC for about an hour and then to  
2700EC. After 30-60 minute heating under atmospheric  
pressure, the heating is continued under vacuum for about  
20 an hour. The polymer is then cooled under nitrogen and  
recovered.

EXAMPLE 19  
COPOLYMERIZATION OF COMPOUND 27 WITH TETRAMETHYLENE GLYCOL  
25 AND ISOPHTHALATE

A three necked flask equipped with a nitrogen inlet  
tube extending below the surface of the reaction mixture, a  
mechanical stirrer, and an exit tube for nitrogen and  
30 evolved hydrogen chloride is flushed with nitrogen and  
charged first with a isophthaloyl chloride followed by a  
stoichiometric amount of a mixture of tetramethylene glycol  
and Compound 27 ligand. The heat of reaction would cause  
the isophthaloyl chloride to melt. The reaction is stirred



vigorously and nitrogen is passed through the reaction mixture to drive away the hydrogen chloride (and collected in an external trap). The temperature of the reaction is then raised to 180°C and held at that temperature for 1 hour. During the last 10 minutes of the 180°C heating cycle, the last of the hydrogen chloride is removed by reducing the pressure to 0.5 - 1.0 mm. The copolymer is obtained as a white solid. Compound 27 in the polymer backbone is then complexed with manganese chloride.

10

## EXAMPLE 20

## ADMIXTURE OF COMPOUND 38 WITH POLYPROPYLENE

Compound 38 was determined to be thermally stable up to 350EC. 0.105 g of Compound 38 was added to 4.9 g of cryoground polypropylene. The mixture was melted at 250EC and extruded into a strand and a fiber. In this manner, a polypropylene modified with a non-proteinaceous catalyst, 2% by weight, was made. The product strand was cryoground and extracted with pure water. Active Compound 38, as confirmed by both stopped-flow kinetic analysis and HPLC-UV spectroscopy, was extracted from the strand. The concentration of Compound 38 in the water has been calculated to correspond to approximately a 10% elution of the admixed Compound 38 from the cryoground polymer. This suggests that the polypropylene would release active PACPeD catalyst at the plastic-human body tissue interface where it would serve to reduce inflammation. Other polymers which melt under 300EC and which would be suitable for use in the above process (with any appropriate temperature changes) are polyethylene, polyethylene terephthalate, and polyamides.

30

## EXAMPLE 21

IN VIVO EVALUATION OF THE INFLAMMATORY RESPONSE TO  
SEVERAL SURFACE COVALENTLY CONJUGATED POLYMERS AND METAL

5        Samples of biomaterials, with and without PACPeD  
catalysts, in the form of 5 - 6 mm discs were implanted  
subcutaneously on the dorsal surface of female, 250 - 300  
gm, Sprague Dawley rats. All disks were sterilized by  
three brief rinses in 70% alcohol followed by five brief  
10 rinses in sterile saline (0.9% NaCl) just prior to  
implantation. All biomaterials were conjugated with  
Compound 43. Polyurethane implants were bathed in sterile  
saline for one hour prior to sterilization in ethanol and  
implantation. Animals were initially anesthetized with 5%  
15 oxygen and 95% carbon dioxide to shave the dorsal region  
followed by methefane vapor administered through a nose  
cone during surgery. Following a sterile scrub of the  
surgical field, a 5 to 6 cm incision through the skin was  
made along the dorsal midline, a pocket in the interstitial  
20 fascia was prepared with a blunt scissors and the implant  
disks were inserted. The wound was closed with surgical  
staples. All animals were ambulatory within one hour of  
anesthesia. For the polyurethane and polyethylene study,  
each animal received an untreated control and two PACPeD  
25 treated disks at a high and low dose. For the tantalum  
study, each animal received a total of four disks, two  
controls containing to two types of linkers and two matched  
PACPeD treated discs. After periods of 3, 7, 14, and 28  
days, animals were sacrificed with 100% carbon dioxide and  
30 the dorsal skin flap was removed and fixed in 10% neutral  
buffered formalin. The skin tissue was pinned upside down  
for photography of the implants in situ and the individual  
implants with surrounding tissue were excised and processed  
in paraffin for light microscopy. PE and PEUU implants

were sectioned with the implants embedded in the paraffin block. Tantalum implants were embedded in paraffin and the paraffin block was cut in half with a low speed diamond saw. These halves were then cooled in liquid nitrogen and fractured with a cold razor blade to expose the tantalum disc. The disc was then removed from the block leaving the implant capsule intact. The tissue blocks were remelted and mounted to expose the implant capsule for microtomy. Sections were stained with hematoxylin and eosin and Gomori trichrome (Sigma, St. Louis MO). In addition, sections were stained immunohistochemically to identify monocyte-derived macrophages with a macrophage specific antibody, ED1 (Chemicon Inc., Temecula, CA). The cellular composition of the implant capsule and surrounding tissue and the matrix composition were scored visually. Measurements of foreign body giant cells number and capsule thickness were made by visual inspection and by computer based measurement of digital micrographs. All data were reported as the mean and standard deviation.

## Results

### Conjugated Polyethylene

Histological analysis was performed on triplicate sets of untreated control PE disks and two PACPeD treated PE disks having with either a low (0.06 %) or high (1.1 % (w/w)) level of PACPeD after 3, 7, 14 and 28 days of implantation. These times were selected in order to observe the acute inflammation phase and the progression to a chronic inflammation. Although differences in the healing response were observed at each time, major differences were apparent at 3 and 28 days. At 3 days, control PE disks were completely surrounded by a dense granulation tissue consisting of neutrophils and macrophages, Figure 5A. Small blood vessels in tissue

adjacent to the implant contained many adherent monocytes and leukocytes and some in various stages of transendothelial migration from blood to implant tissue. In striking contrast, the granulation tissue surrounding  
5 low and high dose PACPeD - PE, Figures 5B and 5C, contained very few and no neutrophils, respectively. Numerous macrophages were present on the low dose implant and labeled with ED1 antibody to suggest that they are monocyte derived. In the high dose implant capsule, the  
10 number of macrophages was greatly reduced and fibroblast like cells constituted the major cell type. In addition, blood vessels adjacent to the PACPeD-PE implants contained no adherent leukocytes or monocytes.

After 28 days equally remarkable differences were  
15 observed. In the control, foreign body giant cells (FBGCs) formed a layer between the implant and the implant capsule tissue, Figure 6A, to indicate that chronic inflammation was underway. FBGCs also filled the many scratches that formed the rough PE surface. The implant capsule tissue  
20 consisted of layered fibroblasts, some ED1 positive macrophages, a few neutrophils and collagen matrix. For the low level PACPeD disks, the capsule had a marked reduction in FBGCs on the surface and in number of cells in the capsule in comparison to control, Figure 6B. With the  
25 high PACPeD-PE disks, FBGCs were rarely observed, Figure 6C. The number of FBGCs observed in two independent sections per disk for a total of six counts per treatment group were averaged and revealed a statistically significant decrease in FBGC with PACPeD-PE over control,  
30 Figure 7. In addition, the thickness of the implant capsule as measured from the same sections was significantly reduced in comparison to untreated control PE.

### Polyurethane

Histological analysis was performed on triplicate sets of untreated control PEUU disks and two PACPeD treated PE disks having with either a low (0.6 %) or high (3.0 % w/w) level of PACPeD after 3, 7, 14 and 28 days of implantation. Although, PEUU is well known to be less inflammatory than polyethylene, the effect of surface bound PACPeD mimic was obvious and similar to that observed for PE disks at 3 and 28 days. At 3 days, implant capsules of control PEUU disks contained neutrophils and ED1 positive macrophages although their numbers were estimated to be two orders of magnitude less than PE control. Capsules surrounding the low level PACPeD - PEUU implants had a markedly reduced but detectable number of neutrophils with macrophages and fibroblast being predominant. As was observed for the PACPeD-PE implants, capsule tissue around the high dose PACPeD-PEUU disks contained no observable neutrophils and a reduced number of macrophages.

At 28 days, implant capsules around the PEUU control disks had a layer of adherent FBGCs and layers of fibroblasts, ED1 positive macrophages and collagen matrix, Figure 8A. With the low level PACPeD, Figure 8B, the number of FBGCs was reduced although the implant capsule contained fibroblast and fewer ED1 positive macrophages and had a thickness similar to the control. The high level PACPeD-PEUU disk capsule had very few FBGCs and the capsule thickness was estimated to be one half of the control capsule, Figure 8C.

It is well known that PEUU is susceptible to biodegradation *in vivo* leading to the formation of surface pits and cracks. To monitor this effect in control and functionalized disks, scanning electron microscopy, SEM, was used to examine non-implanted disks and 28 day implanted disks, Figures 1-3. The non-implanted PEUU film

showed a smooth surface with no cracks or pitted areas, Figure 1. The implanted control PEUU sample after 28 days contained large, multiple cracks and areas where the surface had been eroded, Figure 2. The implanted PACPeD-  
5 PEUU sample showed no obvious differences compared to the non-implanted control, Figure 3. Hence, in addition to inhibiting both acute and chronic inflammatory responses, PACPeD linked to PEUU surface inhibited surface degradation observed at 28 days.

10

#### Tantalum

Tantalum disks treated with either the silane linker or the PACPeD and silane linker were implanted for 3 and 28 days. The healing response was similar to that seen  
15 for treated and untreated polymers. After 3 days, a neutrophil rich granulation tissue enveloped the Ta-silane linker treated disk, figure 9A. With PACPeD treatment, the neutrophils were absent with macrophages and matrix making up the bulk of the implant bed, figure 9B. After  
20 28 days the control disks had a more pronounced implant capsule which was reduced in thickness at PACPeD treated disks, Figure 10.

EXAMPLE 22  
IN VIVO EVALUATION OF THE INFLAMMATORY RESPONSE TO  
POLYPROPYLENE ADMIXED WITH COMPOUND 54

5    Sample Fiber Preparation

      The polypropylene implants for the rat studies were made in a fiber form. After a dry blend was made in the cryo-grinder, the mixture was subjected to twin screw mixing in a DACA melt mixer. 3 gms of PP and 60 mg of  
10    Compound 54 (more lipophilic than Compound 38) was used. The impact time in the cryogrinder was 5 minutes. The melt mixing chamber was held at 250°C. The mixing time was 5 minutes with the rpm being 50. No appreciable differences in the torque was seen between the control and the Compound  
15    54 incorporated PP.

      A 50 denier fiber with 30% of elongation to break was the target. The parameters in the DACA melt spin equipment were the following:

Diameter of the spinneret:	0.5 mm
20    Piston speed:	9.82 mm/min
Spin speed of the main godet:	1      2.85 RPM
Draw ratio:	7
Temperature of the plate:	125°C
Temperature of the barrel:	250°C

25

      The extruded strands from the melt blending were cut into little pieces which fed into the barrel more easily. The melt spinning was done at 250°C. Because the medical grade polymer degrades after 20 minutes at high temperature  
30    we had to use a flow rate of 0.35g/min (the amount of PP in the barrel is 7g).

Implantation Procedure

Polypropylene fibers, with and without Compound 54 mimic, were implanted subdermally in 250 - 300 gram female rats. The polypropylene fiber implant consisted of a 15 to 20 cm length that was wrapped and tied into a figure eight shape measuring about 2 cm by 0.5. Animals were anesthetized with a mixture of 50/10 mg/kg Ketamine/Xylazine by intraperitoneal injection. The right flank was shaved and scrubbed with surgical scrub. A small 1.5 cm long incision was made over the right haunch. A subcutaneous pocket was made and the appropriate piece of material was placed in the pocket. The implants were briefly rinsed in 70% alcohol and rinsed with 2 dips in sterile saline prior to insertion into the tissue pocket. The incision was closed with a stainless steel staple. The rats were returned to their cages for recovery.

The animal were removed from their cages after 21 days post implant and sacrificed by CO<sub>2</sub> inhalation. The implants were removed with overlying skin attached and fixed in Streck STF fixative overnight at 4-8°C. The explants were cut into two or three pieces to expose polymer cross-sections and were processed for embedding in paraffin. Routine sections were cut and stained with hematoxylin and eosin or Masson Trichrome and immunostained with an antibody specific of macrophages, EDI (Chemicon Inc.).

#### In Vivo Response to Implanted Compound 54 Containing Polypropylene

Gross histology examination of control PP fibers attached to the under surface of the skin flap explants evidenced the fibers to be surrounded by a relatively thick matrix of collagen. The position and overall shape of the implant were discernible but individual fibers could not be seen. Histological cross-sections confirmed a relatively thick wrap of connective tissue. In addition to matrix,



higher magnification views reveal an intense inflammatory reaction at each fiber. Control fibers cover with one to two layers of cells which appear to be macrophages based on positive immunohistochemical staining with the rat  
5 macrophage marker, ED1, Figure 4A. In addition, foreign body giant cells were also present on all control fibers. These observations are consistent with the expected chronic inflammatory response.

Compound 54 containing PP fibers exhibited a different  
10 response. Gross examination reveal an implant site in which the individual fibers were clearly visible. It was obvious that the fibrotic and cellular response which covered the control PP fibers was reduced. Histologically, a reduced fibrotic response was apparent, with only a thin  
15 wrap of matrix being observed in Trichrome stained sections. In addition, the inflammatory response at individual SODm containing fibers was markedly reduced. Typically, modified fibers were covered by a thin layer of matrix and few fibroblasts and only partial coverage by  
20 macrophages, Figure 4B. Foreign body giant cells were seldom observed on modified fibers. A count of foreign body giant cells per fiber were performed on control and Compound 54 containing PP fibers. Control fiber FBGC counts were  $2.63 \pm 1.34$  per fiber,  $n = 20$  while modified  
25 fibers had  $1.28 \pm 1.04$  FBGC per fiber,  $n = 40$ .

Despite the striking difference in the inflammatory response, the number or density of fine capillaries appeared to be very similar between the control and modified fibers. This was assessed visually in tissues  
30 spaces between the fibers within the hank and the tissue surrounding the hank implant.

EXAMPLE 23  
LUMINOL ANALYSIS OF MODIFIED POLYMERS AND METALS  
TO DETERMINE  
SUPEROXIDE DISMUTATING ACTIVITY

5

The Michelson assay uses xanthine oxidase and hypoxanthine to produce superoxide radical anion in situ in a steady-state manner. If not eliminated from the solution with an antioxidant, superoxide then reacts with luminol to produce a measurable amount of light. This reaction is stoichiometric and provides a linear response under pseudo first-order reaction conditions (i.e.  $[\text{luminol}] \gg [\text{O}_2^-]$ ). The light emission is measured over several minutes (as the enzyme-substrate solution produces superoxide at a specific rate) and the integration of units over that time is reported. It should then be possible to take samples of antioxidants and determine the presence of catalyst, the rate of dismutation, and/or whether the compound is actually catalytic or stoichiometric in its ability to dismute superoxide.

Using this method, we have taken sample films<sup>1/</sup> p. 74. (lactide/ glycolide polymer) doped with Compound 38, a known catalyst for the dismutation of superoxide and the parent compound in our current SAR, and analyzed them on a Turner Designs TD-20/20 Luminometer.<sup>2/</sup> unpublished results. 400uL of a 0.05 unit/mL xanthine oxidase, 0.1 mM EDTA and 0.1 mM Luminol in 0.1 M glycine buffer at pH 9; 200uL of a

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<sup>1/</sup> Michelson, A.M. In Handbook of Methods for Oxygen Radical Research, Greenwald, R.A., Ed.; CRC:Boca Raton, 1989;

<sup>2/</sup> Gary W. Franklin; Monsanto Notebook, p. 6136376,

250 uM xanthine solution are added via autoinjector to a one 2 square millimeter sample of each film in the sample well. The sample is then run on the Luminometer, and the reading translated into an integration. Samples of PEUU  
5 surface covalently conjugated with Compound 43 were tested and found to possess superoxide dismutating activity.

#### EXAMPLE 24 STOPPED-FLOW KINETIC ANALYSIS

10

Stopped-flow kinetic analysis has been utilized to determine whether a compound can catalyze the dismutation of superoxide (Riley, D. P., Rivers, W. J. and Weiss, R. H., "Stopped-Flow Kinetic Analysis for Monitoring  
15 Superoxide Decay in Aqueous Systems," Anal. Biochem, 196: 344-349 1991). For the attainment of consistent and accurate measurements all reagents were biologically clean and metal-free. To achieve this, all buffers (Calbiochem) were biological grade, metal-free buffers and were handled  
20 with utensils which had been washed first with 0.1N HCl, followed by purified water, followed by a rinse in a  $10^{-4}$  M EDTA bath at pH 8, followed by a rinse with purified water and dried at 65.degree. C. for several hours. Dry DMSO solutions of potassium superoxide (Aldrich) were prepared  
25 under a dry, inert atmosphere of argon in a Vacuum Atmospheres dry glovebox using dried glassware. The DMSO solutions were prepared immediately before every stopped-flow experiment. A mortar and pestle were used to grind the yellow solid potassium superoxide (.about.100 mg). The  
30 powder was then ground with a few drops of DMSO and the slurry transferred to a flask containing an additional 25 ml of DMSO. The resultant slurry was stirred for 1/2 h and then filtered. This procedure gave reproducibly .about.2 mM concentrations of superoxide in DMSO. These solutions were

transferred to a glovebag under nitrogen in sealed vials prior to loading the syringe under nitrogen. It should be noted that the DMSO/superoxide solutions are extremely sensitive to water, heat, air, and extraneous metals. A  
5 fresh, pure solution has a very slight yellowish tint.

Water for buffer solutions was delivered from an in-house deionized water system to a Barnstead Nanopure Ultrapure Series 550 water system and then double distilled, first from alkaline potassium permanganate and  
10 then from a dilute EDTA solution. For example, a solution containing 1.0 g of potassium permanganate, 2 liters of water and additional sodium hydroxide necessary to bring the pH to 9.0 were added to a 2-liter flask fitted with a solvent distillation head. This distillation will oxidize  
15 any trace of organic compounds in the water. The final distillation was carried out under nitrogen in a 2.5-liter flask containing 1500 ml of water from the first still and  $1.0 \times 10^{-6}$  M EDTA. This step will remove remaining trace metals from the ultrapure water. To prevent EDTA mist from  
20 volatilizing over the reflux arm to the still head, the 40-cm vertical arm was packed with glass beads and wrapped with insulation. This system produces deoxygenated water that can be measured to have a conductivity of less than 2.0 nanomhos/cm<sup>2</sup>.

25 The stopped-flow spectrometer system was designed and manufactured by Kinetic Instruments Inc. (Ann Arbor, Mich.) and was interfaced to a MAC IICX personal computer. The software for the stopped-flow analysis was provided by Kinetics Instrument Inc. and was written in QuickBasic with  
30 MacAdios drivers. Typical injector volumes (0.10 ml of buffer and 0.006 ml of DMSO) were calibrated so that a large excess of water over the DMSO solution were mixed together. The actual ratio was approximately 19/1 so that the initial concentration of superoxide in the aqueous

solution was in the range 60-120  $\mu$ M. Since the published extinction coefficient of superoxide in  $H_2O$  at 245 nm is about  $2.25 \times 10^4 M^{-1} cm^{-1}$  (1), an initial absorbance value of approximately 0.3-0.5 would be expected for a 2-cm path length cell, and this was observed experimentally. Aqueous solutions to be mixed with the DMSO solution of superoxide were prepared using 80 mM concentrations of the Hepes buffer, pH 8.1 (free acid+Na form). One of the reservoir syringes was filled with 5 ml of the DMSO solution while the other was filled with 5 ml of the aqueous buffer solution. The entire injection block, mixer, and spectrometer cell were immersed in a thermostated circulating water bath with a temperature of  $21^\circ C \pm 0.5^\circ C$ . Prior to initiating data collection for a superoxide decay, a baseline average was obtained by injecting several shots of the buffer and DMSO solutions into the mixing chamber. These shots were averaged and stored as the baseline. The first shots to be collected during a series of runs were with aqueous solutions that did not contain catalyst. This assures that each series of trials were free of contamination capable of generating first-order superoxide decay profiles. If the decays observed for several shots of the buffer solution were second-order, solutions of manganese(II) complexes could be utilized. In general, the potential SOD catalyst was screened over a wide range of concentrations. Since the initial concentration of superoxide upon mixing the DMSO with the aqueous buffer was about  $1.2 \times 10^{-4} M$ , we wanted to use a manganese (II) complex concentration that was at least 20 times less than the substrate superoxide. Consequently, we generally screened compounds for superoxide dismutating activity using concentrations ranging from  $5 \times 10^{-7}$  to  $8 \times 10^{-6} M$ . Data acquired from the experiment was imported into a suitable math program (e.g., Cricket Graph) so that

standard kinetic data analyses could be performed. Catalytic rate constants for dismutation of superoxide by manganese(II) complexes were determined from linear plots of observed rate constants ( $k_{\text{obs}}$ ) versus the concentration of the manganese(II) complexes.  $k_{\text{obs}}$  values were obtained from linear plots of  $\ln$  absorbance at 245 nm versus time for the dismutation of superoxide by the manganese(II) complexes.

#### EXAMPLE 25

USE OF HYALURONIC ACID ESTERS SURFACE COVALENTLY CONJUGATED WITH COMPOUND 43 TO PRODUCE A NEURAL GROWTH GUIDE CHANNEL DEVICE

A guide channel with a composite thread/polymeric matrix structure wherein the thread comprises HYAFF 11 (total benzyl ester of HY, 100% esterified) and the matrix is composed of HYAFF 11p75 (benzyl ester of HY 75% esterified) is obtained by the following procedure.

##### A. Preparation of Esters

Preparation of the Benzyl Ester of Hyaluronic Acid (HY): 3 g of the potassium salt of HY with a molecular weight of 162,000 are suspended in 200 ml of dimethylsulfoxide; 120 mg of tetrabutylammonium iodide and 2.4 g of benzyl bromide are added. The suspension is kept in agitation for 48 hours at 30E C. The resulting mixture is slowly poured into 1,000 ml of ethyl acetate under constant agitation. A precipitate is formed which is filtered and washed four times with 150 ml of ethyl acetate and finally vacuum dried for twenty four hours at 30E C. 3.1 g of the benzyl ester product in the title are obtained. Quantitative determination of the ester groups is carried out according to the method described on pages 169-

172 of Siggia S. and Hanna J. G. "Quantitative organic Analysis Via Functional Groups," 4th Edition, John Wiley and Sons.

Preparation of the (Partial) Benzyl Ester of  
5 Hyaluronic Acid (HY)-75% Esterified Carboxylic Groups, -25 %  
Salified Carboxylic Groups (Na): 12.4 g of HY  
tetrabutylammonium salt with a molecular weight of 170,000,  
corresponding to 20 m.Eq. of a monomeric unit, are  
10 solubilized in 620 ml of dimethylsulfoxide at 25E C. 120  
mg of tetrabutylammonium iodide and 15.0 m.Eq. of benzyl  
bromide are added and the resulting solution is kept at a  
temperature of 30E for 12 hours. A solution containing 62  
ml of water and 9 g of sodium chloride is added and the  
resulting mixture is slowly poured into 3,500 ml of acetone  
15 under constant agitation. A precipitate is formed which is  
filtered and washed three times with 500 ml of  
acetone/water, 5:1, and three times with acetone, and  
finally vacuum dried for eight hours at 30E C.

The product is then dissolved in 550 ml of water  
20 containing 1% sodium chloride and the solution is slowly  
poured into 3,000 ml of acetone under constant agitation.  
A precipitate is formed which is filtered and washed twice  
with 500 ml of acetone/water, 5:1, three times with 500 ml  
of acetone, and finally vacuum dried for 24 hours at 30E C.  
25 7.9 g of the partial propyl ester compound in the title are  
obtained. Quantitative determination of the ester groups is  
carried out using the method of R. H. Cundiff and P. C.  
Markunas Anal. Chem. 33, 1028-1030, (1961).

The HYAFP esters are then surface covalently  
30 conjugated with Compound 43 as in Example 14.

#### B. Production of the Device

A thread of total HYAFP 11 esters, 250 denier, with a  
minimum tensile strength at break of 1.5 gr/denier and 19%

elongation is entwined around an electropolished AISI 316 steel bar with an outer diameter of 1.5 mm, which is the desired inner diameter of the composite guide channel. The woven product is obtained using a machine with 16 loaders per operative part.

A typical tube-weaving system (like the one shown in U.S. Pat. No. 5,879,359) comprising the steel bar with a threaded tube fitted over it is placed in position. The apparatus is rotated at a speed of 115 rpm. A quantity of HYAFF 11p75/dimethylsulfoxide solution at a concentration of 135 mg/ml is spread over the rotating system. The excess solution is removed with a spatula, and the system is removed from the apparatus and immersed in absolute ethanol. After coagulation, the guide channel is removed from the steel bar and cut to size.

The channel made by the above technique is 20 mm long, 300  $\mu\text{m}$  thick, has an internal diameter of 1.5 mm, and has a weight of 40 mg, equal to 20 mg/cm.

#### EXAMPLE 26

##### USE OF METALS SURFACE COVALENTLY CONJUGATED WITH COMPOUND 43 TO PRODUCE A STENT

A stent may be formed from surgical stainless steel alloy wire which is bent into a zigzag pattern, and then wound around a central axis in a helical pattern. Referring now more particularly to Figures 11-17, there is illustrated in FIG. 11 a midpoint in the construction of the stent which comprises the preferred embodiment of the present invention. FIG. 11 shows a wire bent into an elongated zigzag pattern 5 having a plurality of substantially straight wire sections 9-15 of various



lengths separated by a plurality of bends 8. The wire has first and second ends designated as 6 and 7, respectively. Zigzag pattern 5 is preferably formed from a single strand of stainless steel wire having a diameter in the range of 0.005 to 0.025 inch.

FIG. 13 shows a completed stent 30. The construction of the stent is completed by helically winding elongated zigzag pattern 5 about a central axis 31. Zigzag pattern 5 is wound in such a way that a majority of the bends 8 are distributed in a helix along the length of the stent 30. There are preferably about twelve interconnected bends in each revolution of the helix, or six adjacent bends of the zigzag pattern in each revolution. The construction of stent 30 is completed by interconnecting adjacent bends of the helix with a filament 32, preferably a nylon monofilament suture. Filament 32 acts as a limit means to prevent the stent from further radial expansion beyond the tubular shape shown in FIGS. 13 and 14. The tubular shape has a central axis 31, a first end 33 and a second end 35. Each end of stent 30 is defined by a plurality of end bends 36, which are themselves interconnected with a filament 34. Other embodiments of the present invention are contemplated in which the end bends 36 are left unconnected in the finished stent. FIG. 14 shows an end view of stent 30 further revealing its tubular shape. FIG. 15 shows stent 30 of FIG. 13 when radially compressed about central axis 31 such that the straight wire sections and the bends are tightly packed around central axis 31.

Referring back to FIG. 11, the zigzag pattern is made up of straight wire sections having various lengths which are distributed in a certain pattern to better facilitate the helical structure of the final stent construction. For instance, in one embodiment, end wire sections 9 could be made to a length of 9 mm followed by two wire sections 11

each being 11 mm in length. Wire sections 11 are followed by two 13 mm wire sections 13, which are in turn followed by two wire sections 15 having a length of 15 mm. Sections 15 are followed by a single wire section 17 having a length of 17 mm. These gradually increasing wire sections at either end of the zigzag pattern enable the final stent to have well defined square ends. In other words, the gradually increasing length wire sections on either end of the zigzag pattern enable the final stent to have a tubular shape in which the ends of the tube are substantially perpendicular to the central axis of the stent. Following wire section 17, there are a plurality of alternating length sections 13 and 15. Short sections 13 being 13 mm in length and long sections 15 being 15 mm in length. This alternating sequence is continued for whatever distance is desired to correspond to the desired length of the final stent. The difference in length between the short sections 13 and long sections 15 is primarily dependent upon the desired slope of the helix (see .beta. in FIG. 16) and the desired number of bends in each revolution of the helix.

FIG. 16 is an enlarged view of a portion of the stent shown in FIG. 13. The body of stent 30 includes a series of alternating short and long sections, 13 and 15 respectively. A bend 8 connects each pair of short and long sections 13 and 15. Each bend 8 defines an angle  $2\angle$  which can be bisected by a bisector 40. These short and long sections are arranged in such a way that bisector 40 is parallel to the central axis 31 of the stent. This allows the stent to be radially compressed without unnecessary distortion.

FIG. 12 shows an enlarged view of one end of the zigzag pattern. End 6 of the wire is bent to form a closed eye portion 20. Eye 20 is preferably kept closed by the application of the small amount of solder to the end 6 of

the wire after it has been bent into a small loop. Each of the bends 8 of the zigzag pattern are bent to include a small eye portion designated as 21 and 23 in FIG. 12, respectively. Eye 21 includes a small amount of solder 22 which renders eye 21 closed. Eye 23 includes no solder and is left open. The bends 8 which define the helix can be either in the form of a closed eye, as in eye 21, or open as in eye 23.

After forming the stent, the stent is then modified by surface covalent conjugation with a silyl linker, as in Example 13. By treatment with acid mixtures well known in the art, the stainless steel surface can be oxidized to display a layer of hydroxide. The conjugation then proceeds as in Example 13.

#### EXAMPLE 27

##### USE OF SURFACE COVALENTLY CONJUGATED PET FIBERS TO PRODUCE A WOVEN VASCULAR GRAFT

PET fibers are surface covalently conjugated with Compound 43 according to Example 7. The vascular graft fabric is formed from single ply, 50 denier, 47 filament (1/50/47) pretexturized, high shrinkage (in excess of approximately 15%), polyethylene terephthalate (PET) yarns woven in a plain weave pattern with 83 ends/inch and 132 picks/inch (prior to processing). The vascular graft fabric, prior to processing, has a double wall size of less than 0.02 inches and preferably has a double wall thickness of about 0.01 inches. The yarns may be twisted prior to weaving and a graft with 8 twists per inch has provided acceptable properties. Other weave patterns, yarn sizes (including microdenier) and thread counts also are contemplated so long as the resulting fabric has the

desired thinness, radial compliance and resistance to long term radial dilation and longitudinal expansion.

5 The woven fabric is washed at an appropriate temperature, such as between 60E-90E C, and then is steam set over a mandrel to provide the desired tubular configuration. The graft is then dried in an oven or in a conventional dryer at approximately 150E F. Any of the washing, steaming and drying temperatures may be adjusted to affect the amount of shrinkage of the fabric yarns. In 10 this manner, the prosthetic is radially compliant to the extent necessary for the ends of the graft to conform to the slightly larger anchoring sections of the aorta, but resists radial dilation that otherwise could lead to rupture of the aneurysm and axial extension that could 15 block the entrance to an iliac artery. Radial dilation is considered to occur when a graft expands a further 5% after radial compliance. The 5% window allows for slight radial expansion due to the inherent stretch in the yarn of the fabric.

20 The thin walled, woven vascular graft fabric is be formed into a tubular configuration and collapsed into a reduced profile for percutaneous delivery of the prosthetic to the delivery site. The implant is sufficiently resilient so that it will revert back to its normal, expanded shape 25 upon deployment either naturally or under the influence of resilient anchors that secure the implant to the vessel wall, and or, alternatively, struts that prevent compression and twisting of the implant. The thin wall structure allows small delivery instruments (18 Fr or 30 smaller) to be employed when the graft is percutaneously placed. The fine wall thickness also is believed to facilitate the healing process. The graft, when used for the repair of an abdominal aortic aneurysm, may be provided

in a variety of outer diameters and lengths to match the normal range of aortic dimensions.

The biologically compatible prosthetic fabric encourages tissue ingrowth and the formation of a neointima lining along the interior surface of the graft, preventing clotting of blood within the lumen of the prosthetic which could occlude the graft. The graft has sufficient strength to maintain the patency of the vessel lumen and sufficient burst resistance to conduct blood flow at the pressures encountered in the aorta without rupturing. The graft is usually preclotted with either the patient's own blood or by coating the fabric with an impervious material such as albumin, collagen or gelatin to prevent hemorrhaging as blood initially flows through the graft. Although a constant diameter graft is preferred, a varying dimensioned prosthetic also is contemplated. The graft is also usually provided with one or more radiopaque stripes to facilitate fluoroscopic or X-ray observation of the graft.

EXAMPLE 28  
USE OF COPOLYMERIZED POLYURETHANE TO  
TO INSULATE CARDIAC SIMULATOR LEAD WIRE

5        A die-clad composite conductor is made with a highly  
conducting core and a cladding layer. Copper and copper  
alloys are particularly suitable for the core material of  
the composite conductor. Pure copper is preferable, but  
alloys such as  $\text{Cu}_{0.15}\text{Zr}$ ,  $\text{Cu}_4\text{Ti}$ ,  $\text{Cu}_2\text{Be}$ ,  $\text{Cu}_{1.7}\text{Be}$ ,  $\text{Cu}_{0.7}\text{Be}$ ,  
10  $\text{Cu}_{28}\text{Zn}$ ,  $\text{Cu}_{37}\text{Zn}$ ,  $\text{Cu}_6\text{Sn}$ ,  $\text{Cu}_8\text{Sn}$  and  $\text{Cu}_2\text{Fe}$  may be used. A  
metal selected from the group consisting of tantalum,  
titanium, zirconium, niobium, titanium-base alloys,  
platinum, platinum-iridium alloys, platinum-palladium  
alloys and platinum-rhodium alloys is applied as a cladding  
15 layer to the conducting core by drawing through a die. The  
cladding layer thickness is between 0.0025 and 0.035 mm,  
while the core diameter is between 0.04 and 0.03 mm.  
Although a single strand conductor could be used, the risks  
of breakage are reduced and the conductivity is increased  
20 without going beyond the above described preferred ranges  
for core diameter and cladding if a stranded conductor is  
used. Furthermore a stranded conductor provides increased  
flexibility. Thus, it is preferred that the conductor in  
the cable be composed of two or more thinner strands  
25 twisted together.

      The clad wire conductor is enclosed in an elastic  
covering tube, which consists of a synthetic elastomer such  
as flexible polyurethane. A polyurethane which has been  
copolymerized with a PACPeD catalyst, such as the  
30 polyurethane of Example 12, should be used. It is  
sufficiently elastic and flexible to make possible its  
introduction into the heart chamber simply by being carried  
along through the blood stream. The biocompatibility of the  
clad wire conductor of the cable is improved by oxidizing

the surface of the clad wire and covalently conjugating a PACPeD catalyst to the wire, as in Example 13.